CERI

Receipt Number	822-17-D-4273
Study Number	A16-0822

FINAL REPORT

Acute Oral Toxicity Study of APFHx in Rats

May, 2018

Chemicals Evaluation and Research Institute, Japan, Hita

GLP STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor DAIKIN INDUSTRIES, LTD.

Title Acute Oral Toxicity Study of APFHx in Rats

Study Number A16-0822

The study was conducted in compliance with the following GLP principles.

OECD Principles of Good Laboratory Practice, November 26, 1997, ENV/MC/CHEM (98)17

This final report accurately reflects the raw data and the test data are valid.

Study Director: May 9, 2018
Date

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1. TITLE

Acute Oral Toxicity Study of APFHx in Rats

2. SPONSOR

Name DAIKIN INDUSTRIES, LTD.

Address 1-1, Nishi Hitotsuya, Settsu-shi, Osaka 566-8585, Japan

3. TESTING FACILITY

Name Chemicals Evaluation and Research Institute, Japan, Hita (CERI Hita)

Address 3-822, Ishii-machi, Hita-shi, Oita 877-0061, Japan

4. OBJECTIVE

The objective of this study is to evaluate the acute oral toxicity of APFHx in rats originating from the oral administration of a single dose and to classify the test substance according to Globally Harmonized System of Classification and Labelling of Chemicals (GHS).

5. TEST METHOD

OECD Guideline for the Testing of Chemicals, No. 420, Acute Oral Toxicity-Fixed Dose Procedure, December 17, 2001

6. GLP PRINCIPLE

OECD Principles of Good Laboratory Practice, November 26, 1997, ENV/MC/CHEM (98)17

7. ANIMAL WELFARE

This study was complied with the guideline for the animal experiment in the testing facility which referred to the following acts and guidelines.

a) Act on Welfare and Management of Animals (Japan, Act Number 105, 1973)
b) Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Ministry of the Environment, Japan, 2006)
c) Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Health, Labour and Welfare (Ministry of Health, Labour and Welfare, Japan, 2006)
d) Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Agriculture, Forestry and Fisheries (Ministry of Agriculture, Forestry and Fisheries, Japan, 2006)
e) Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Ministry of Education, Culture, Sports,

Science and Technology, Japan, 2006)

f) Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006)

8. DATES

Study initiation	March 16, 2018
Animal receipt	March 27, 2018
Administration of 1st sighting study (experiment start)	April 3, 2018
Administration of 2nd sighting study	April 5, 2018
Necropsy of 2nd sighting study	April 6 2018
Administration of main study	April 10, 2018
Necropsy of 1st sighting study	April 17, 2018
Necropsy of main study (experiment completion)	April 24, 2018
Study completion	May 9, 2018

9. PERSONNEL CONCERNED WITH STUDY

Study Director

Responsible scientist

(Responsible for the animal examinations: quarantine, acclimation, care and management of animals, preparation of dosing formulation, administration, clinical observations and measurement of body weights)

Scientist in charge for pathological examination

(Responsible for the pathological examinations)

Other study personnel

(Animal examinations)

(Pathological examinations)

10. RETENTION OF TEST SUBSTANCE, RAW DATA, ETC.

The original study plan, original final report, raw data, study contract documents, test substance information and other record documents will be retained in the testing facility. The remaining test substance will be returned to the sponsor.

The retention period is 10 years after the completion of the study. After the termination of the retention period, any measures (continuous storage, disposal or return) will be done with

the approval of the sponsor.

11. APPROVAL OF FINAL REPORT

Study Director: May 9, 2018

Date

12. SUMMARY

The study was performed according to OECD Guideline for the Testing of Chemicals No. 420 to evaluate the acute oral toxicity of APFHx.

The test substance was dissolved in purified water and administered in a single dose to eight or nine weeks old female Crl:CD(SD) rats by gavage. Clinical signs were observed daily for 14 days and body weights were measured 0 (before administration), 1, 7 and 14 days after the administration. The survived animals were subjected to a gross necropsy 14 days after the administration. The dead animal was subjected to it immediately after it was found dead. The dosages were set at 300 mg/kg for the 1st sighting study and main study and at 2000 mg/kg for the 2nd sighting study. One animal each was used in the sighting studies and four animals were used in the main study.

In the 1st sighting study at 300 mg/kg, no mortalities or moribundities occurred. No abnormalities were observed in the general clinical observation, body weights measurements or necropsy.

In the 2nd sighting study at 2000 mg/kg, one animal was dead. From three hours after the administration, decreased spontaneous locomotion, decreased respiratory rate, incomplete eyelid opening and moist hair (abdomen) were observed. Five hours after the administration, lacrimation was newly observed. The animal was found dead on the next day of the administration. In the necropsy, edematous change of limiting ridge of the forestomach was observed.

In the main study at 300 mg/kg, no mortalities or moribundities occurred. No abnormalities were observed in the general clinical observation, body weights measurements or necropsy.

The hazard class of the acute oral toxicity of APFHx in rats under the tested conditions was classified to "Category 4" of Globally Harmonized System of Classification and Labelling of Chemicals, because no mortalities or moribundities occurred and no evident toxicity was observed in the sighting study and main study at 300 mg/kg, and one animal was dead in the sighting study at 2000 mg/kg.

13. MATERIALS

13.1 Test substance

a) Name, etc. (information provided by the sponsor)

Chemical name 2,2,3,3,4,4,5,5,6,6,6-Undecafluorohexanoic acid, ammonium salt

Other name APFHx

CAS number 21615-47-4

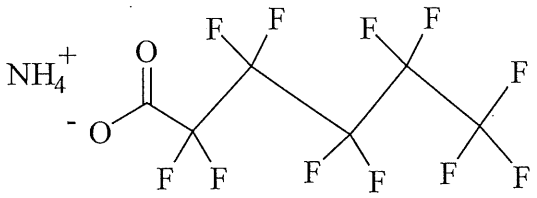
b) Supplier and lot number (information provided by the sponsor)

Supplier DAIKIN INDUSTRIES, LTD.

Lot number C150S1703

c) Structural formula, etc. (information provided by the sponsor)

Structural formula



Molecular formula $C_6H_4F_{11}NO_2$

Molecular weight 331.08

d) Purity, etc. (information provided by the sponsor)

Purity 99.8%

Impurity Water 0.2%

The test substance was treated as 100% in purity.

e) Physicochemical properties (information provided by the sponsor)

Appearance at ordinary temperature White powder

Water solubility >500 g/L

f) Storage conditions

The test substance was put into a shaded and air-tight container and stored in a desiccator in the test substance storage room at room temperature (acceptable range: from 10°C to 30°C).

g) Handling

In order to avoid inhalation and contact with the skin and eyes, chemically resistant gloves, a mask, a head cap, safety glasses and a lab coat were worn when handling the test substance.

13.2 Vehicle

a) Name

Purified water

b) Reason for selection

The test substance was dissolved to purified water at a concentration of 20 w/v%. The condition of the formulation such as color did not change at room temperature four hours

after the preparation. Additionally, purified water is commonly used in the general toxicity study, and the testing facility has the historical control data. Therefore, purified water was selected as a vehicle.

c) Manufacturer, grade, lot number and storage conditions

Manufacturer	Grade	Lot number	Storage place	Storage temperature
Takasugi Pharmaceutical	Japanese Pharmacopoeia	PC171219	Reagent storage room	Room temperature

13.3 Animals

Crl:CD(SD) rats (SPF) were obtained from Charles River Laboratories Japan (Hino Breeding Center). This strain is established as experimental animals and commonly used in the general toxicity study, and the testing facility has the historical control data.

Ten female rats at seven weeks old were obtained and quarantined/acclimatized for six days under group housing of two or three animals per cage. The animals were weighed at the receipt and six days after the receipt. Clinical signs and excretions were observed daily during the quarantine period. No abnormalities were found in the body weights, clinical signs or excretions in any quarantined animals. After the quarantine period, the animals were acclimatized under group housing of three or fewer animals per cage until group allocation. After the group allocation, the animals were housed individually until the administration day. The clinical signs and excretions were observed daily during the acclimation period and no abnormalities were observed in any animals.

The animals were weighed one day before the administration and in the order of the larger weight, one animal each were assigned for the sighting studies and four animals were assigned for the main study.

The animals were identified by painting on the tail using a red oily ink before the group allocation, and by painting on the hair using a yellow aqueous ink for 300 mg/kg and a red aqueous ink for 2000 mg/kg after the group allocation. Cages were identified by labels and a rack was identified by indicating the study number, sex and dose level.

The animals were eight weeks old at the administration of the sighting studies and nine weeks old at that of the main study. Body weights were ranged 189.1 g at the administration of the 1st sighting study, 193.2 g at that of the 2nd sighting study, 196.3 g to 200.0 g at that of the main study. The individual body weights at the administration of the 2nd sighting study and after were confirmed to be within ±20% of the mean animal weights of any previously dosed animals.

13.4 Animal husbandry

The animals were housed in the barrier-system animal rooms (quarantine room 1 and animal room 2) which were maintained from 21 to 25°C, relative humidity of 40 to 70%, 10 to 15 air changes per hour and photoperiod of 12 hours light per day (light on at 7:00 and off at 19:00).

The animals were kept in stainless steel cages with mesh-floor (260W×380D×180H mm) before the group allocation and in stainless steel cages with mesh-floor (165W×300D×150H mm) after the group allocation.

Trays under the cages were changed at the end of the quarantine period and at the group allocation, and changed twice a week after the end of the quarantine period. Feeders, cages and racks were changed at the group allocation.

The animals had free access to a pelleted diet (MF, lot number 171221, Oriental Yeast). Information of the contaminants in the used lots of diets was obtained from supplier and confirmed to meet the requirements in the testing facility which referred to the "Toxic Substances Control Act of US-EPA (1979)".

The diets and housing materials were autoclaved before use at 121°C for 30 minutes.

Chlorinated water in which chloric level maintained from 3 to 5 ppm by adding sodium hypochlorite (Purelox) to Hita City supply water was used as drinking water and the animals also had free access to the water. Contaminants in drinking water were analyzed twice a year, and the results before the receipt of the animals were confirmed to meet the regulations of the "Ordinance on drinking water quality standards" (Ordinance Number 101 of Ministry of Health, Labour and Welfare, Japan).

14. METHODS

14.1 Dose setting

Since no toxicity information about the test substance was available, the dose level of the 1st sighting study was set at 300 mg/kg. The dose levels of the 2nd sighting study and main study were set at 2000 and 300 mg/kg, respectively, according to the test method (Appendices 1 and 2).

14.2 Dose and number of animals etc.

Study	Dose level (mg/kg)	Dosing volume (mL/kg)	Concentration of dosing formulation (w/v%)	Number of animals (Animal number)
1st sighting	300	10	3.00	1 (1)
2nd sighting	2000	10	20.0	1 (2)
Main	300	10	3.00	4 (3-6)

14.3 Dosing formulation

Dosing formulation was prepared on each administration day. The test substance was weighed and mixed with purified water to be dissolved. The solution was filled up to the prescribed volume with purified water to prepare the dosing formulation. The dosing formulation was transferred into a plastic container and carried to the animal room. The dosing formulations were administered within one hour after the preparation.

The weights of the test substance and volume of the dosing formulations are shown below.

Study	Concentration of dosing formulation (w/v%)	Weight of test substance (g)	Volume of formulation (mL)
1st sighting	3.00	0.60036	20
2nd sighting	20.0	4.00062	20
Main	3.00	0.60039	20

14.4 Administration

The animals were fasted for 17 to 19 hours before the administration, and for three to four hours after the administration. The administration was performed once by gavage at 10:10 for the 1st sighting study, at 9:41 for the 2nd sighting study and from 10:27 to 10:29 for the main study.

The administration was conducted with a syringe (TERUMO) and a Nelaton catheter (TERUMO) at the volume of 10 mL/kg based on the body weight measured on the administration day.

14.5 General clinical observation

In the 1st sighting study and main study, the animals were observed continuously for 10 minutes after the administration, and observed 30 minutes and three hours after the administration on the administration day. The animals were observed once in the morning from 1 to 14 days after the administration.

In the 2nd sighting study, the animal was observed continuously for 10 minutes after the administration, and observed 30 minutes, three hours and five hours after the administration on the administration day. The animal was observed once in the morning on the next day of the administration.

14.6 Measurement of body weight

Body weights were measured 0 (before administration), 1, 7 and 14 days after the administration with an electric balance (SARTORIUS).

14.7 Gross necropsy

The survived animals were subjected to a gross necropsy 14 days after the administration. The dead animal was subjected to it immediately after it was found dead. The survived animals were euthanized by bleeding from the abdominal aorta under isoflurane anesthesia. External surface of the body, all orifices, subcutis, cranial, thoracic, abdominal and pelvic cavities with their contents were observed for all the animals.

14.8 Evaluation of result

According to the number of mortalities, moribundities and animals with evident toxicity, the hazard class of an acute oral toxicity of the test substance was classified to the category of GHS (Appendices 1 and 2).

15. DEVIATION FROM STUDY PLAN

No deviation from the study plan occurred.

16. TEST RESULTS

16.1 Clinical signs including mortality

Clinical signs are shown in Table 1.

In the 1st sighting study and the main study at 300 mg/kg, no mortalities or moribundities occurred. No abnormalities were observed in any animals until 14 days after the administration.

In the 2nd sighting study at 2000 mg/kg, one animal was dead. From three hours after the administration, decreased spontaneous locomotion, decreased respiratory rate, incomplete eyelid opening and moist hair (abdomen) were observed. Five hours after the administration, lacrimation was newly observed. The animal was found dead at 8:20 on the next day of the administration.

16.2 Body weights

Body weights are shown in Table 2.

In the 1st sighting study, no abnormalities were observed.

In the main study, the body weight gain of one animal (animal number 3) tended to be low (+4.5 g) one day after the administration.

16.3 Macroscopic findings

Macroscopic findings are shown in Table 3.

In the 1st sighting study and the main study at 300 mg/kg, no abnormalities were observed in any animals.

In the 2nd sighting study at 2000 mg/kg, edematous change of limiting ridge of the forestomach was observed in the dead animal.

17. DISCUSSION AND CONCLUSION

Although no mortalities or moribundities occurred at 300 mg/kg, one animal was dead at 2000 mg/kg. In the dead animal, edematous change of limiting ridge of the forestomach was observed in the necropsy.

The edematous change of limiting ridge of the forestomach was considered to be caused by the irritation of the test substance.

The low tendency of the body weight gain (+4.5 g) observed in one animal in the main study at 300 mg/kg was considered not to be toxicologically significant, because no abnormalities were observed in the general clinical observation or necropsy. Therefore, it was not judged as evident toxicity.

The hazard class of the acute oral toxicity of APFHx in rats under the tested conditions was classified to "Category 4" of GHS, because no mortalities or moribundities occurred and no evident toxicity was observed in the sighting study and main study at 300 mg/kg, and one animal was dead in the sighting study at 2000 mg/kg.

Table 1 Clinical signs

Study	Dose (mg/kg)	Animal No.	Day after administration																	
			0					1	2	3	4	5	6	7	8	9	10	11	12	13	14
			(min.)			(hr.)															
			0[a)]-5	5-10	30	3	5														
1st sighting study	300	1	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
2nd sighting study	2000	2	-	-	-	DS* DRR* IE MH	DS DRR IE MH LA	D													
Main study	300	3	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
		4	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
		5	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
		6	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-

a) Immediately after administration.

-: no abnormalities detected.

DS: decreased spontaneous locomotion, DRR: decreased respiratory rate, IE: incomplete eyelid opening, MH: moist hair (abdomen), LA: lacrimation, D: death.

*: slight.

Table 2 Body weights

Study	Dose (mg/kg)	Animal No.	Body weights (g)			
			Day after administration			
			Initial	1 [a)]	7 [a)]	14 [a)]
1st sighting study	300	1	189.1	209.3 (20.2)	231.5 (22.2)	250.9 (19.4)
2nd sighting study	2000	2	193.2	183.9 [b)] (-9.3)	—	—
Main study	300	3	198.4	202.9 (4.5)	233.2 (30.3)	249.1 (15.9)
		4	196.3	221.2 (24.9)	232.3 (11.1)	240.2 (7.9)
		5	196.8	210.2 (13.4)	225.7 (15.5)	228.6 (2.9)
		6	200.0	214.4 (14.4)	231.8 (17.4)	242.5 (10.7)

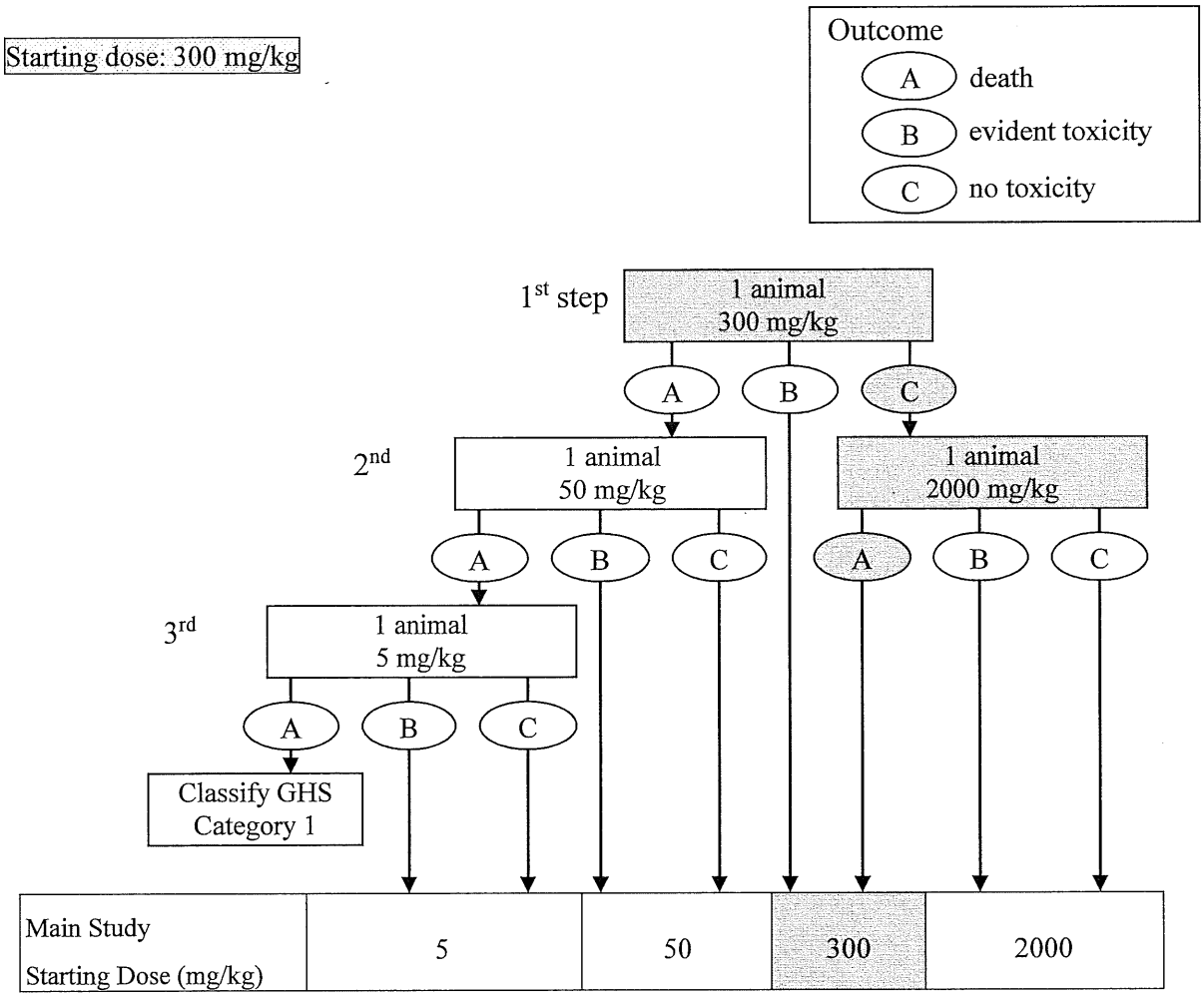
a) Figures in parentheses indicate differences from previous body weight.
b) Dead amimal.

Table 3 Macroscopic findings

Study	Dose (mg/kg)	Animal No.	Fate	Macroscopic findings
1st sighting study	300	1	ss	No abnormalities detected
2nd sighting study	2000	2	d	Forestomach Edematous change of limiting ridge
Main study	300	3	ss	No abnormalities detected
		4	ss	No abnormalities detected
		5	ss	No abnormalities detected
		6	ss	No abnormalities detected

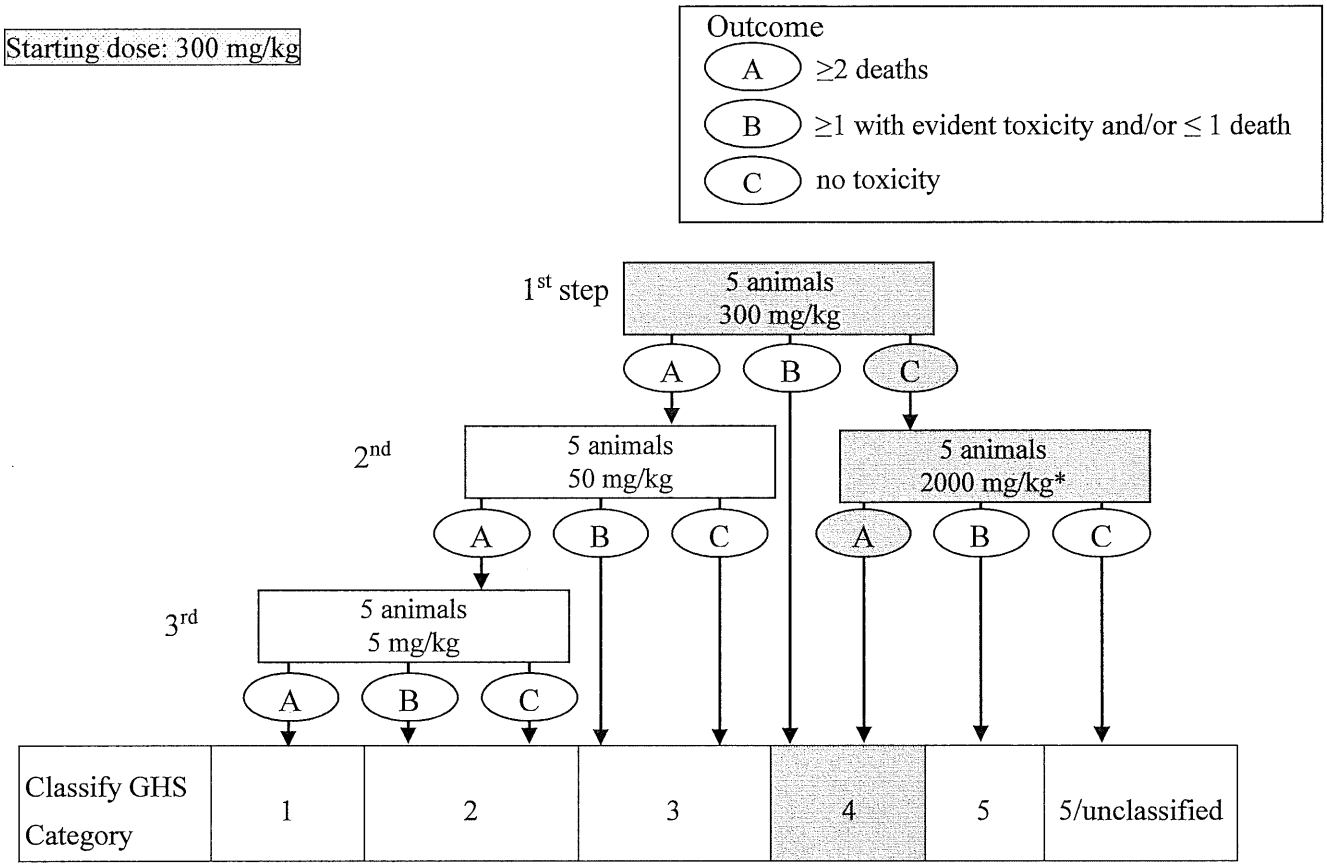
ss: scheduled sacrifice animal, d: dead animal.

Appendix 1 Test procedure for the sighting study described in OECD TG420



The colored cells show the procedure and the result of the sighting study.

Appendix 2 Test procedure for the main study described in OECD TG420



Group size: the 5 animals in each main study group include the animal tested at that dose level in the sighting study.

*: Animal welfare override; since this does level caused death in the sighting study, no further animals were tested. The step directly went to outcome A.

QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: Acute Oral Toxicity Study of APFHx in Rats

Study Number: A16-0822

I assure that the final report accurately describes the test methods and procedures, and that the reported results accurately reflect the raw data of the study. The inspections of this study were carried out and the results were reported to the Study Director and the Test Facility Management by Quality Assurance Unit as follows.

Item of inspection	Date of inspection	Date of report
Study plan	March 19, 2018	March 19, 2018
Preparation of dosing formulations	April 3, 2018	April 3, 2018
Administration and clinical observations	April 3, 2018	April 3, 2018
Allocation and animal identification	April 9, 2018	April 9, 2018
Gross necropsy	April 17, 2018	April 17, 2018
Raw data and draft final report	May 7, 2018	May 7, 2018
Final report	May 9, 2018	May 9, 2018

The inspection result of following item was reported to the Study Director and the Test Facility Management based on the report of facility-based inspection and/or process-based inspection relevant to this study type and timeframe.

Item of inspection	Date of inspection	Date of report
Animal receipt	March 13, 2018	May 9, 2018
Quarantine and acclimatization	March 13, 2018	May 9, 2018
Animal management	March 9, 2018	May 9, 2018
Body weight measurement	March 9, 2018	May 9, 2018

Date: May 9, 2018

Quality Assurance Manager: ____