CERI

Receipt Number	822-16-D-4115
Study Number	A16-0772

FINAL REPORT

Acute Oral Toxicity Study of C6OLF in Rats

March, 2017

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 C6OLF in Rats

Study Number A16-0772

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: March 22, 2017
Date

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1. TITLE

Acute Oral Toxicity Study of C6OLF 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 C6OLF in rats originating from the single oral administration 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 at 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	February 6, 2017
Animal receipt	February 14, 2017
Administration of sighting study (experiment start)	February 21, 2017
Administration of main study	February 23, 2017
Necropsy of sighting study	March 7, 2017
Necropsy of main study (experiment completion)	March 9, 2017
Study completion	March 22, 2017

9. PERSONNEL CONCERNED WITH STUDY

Study Director (Section 2, CERI Hita)

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: March 22, 2017
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 C6OLF.

The test substance was suspended in olive oil including 3 w/v% of polyoxyethylene(20) sorbitan monooleate and administered singly to eight 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 animals were subjected to a gross necropsy 14 days after the administration.

The dosages were set at 2000 mg/kg for sighting study and main study. One animal was used in the sighting study and four animals were used in the main study.

At 2000 mg/kg, no mortalities or moribundities occurred. No abnormalities were observed in the general clinical observation, body weight measurement or necropsy.

Therefore, the hazard class of the acute oral toxicity of C6OLF in rats under the tested conditions was classified to "Category 5 or unclassified" of Globally Harmonized System of Classification and Labelling of Chemicals.

13. MATERIALS

13.1 Test substance

a) Chemical name, etc. (information provided by the sponsor)

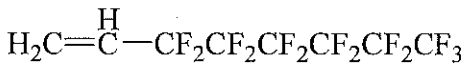
Chemical name	3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooct-1-ene
Other name	C6OLF
CAS number	25291-17-2

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

Supplier	DAIKIN INDUSTRIES, LTD.
Lot number	C2160215

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

Structural formula



Molecular formula	$C_8H_3F_{13}$
Molecular weight	346.09

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

Purity	99.95%	
Impurity	Unknown	0.05%

The test substance was treated as 100% in purity.

e) Physicochemical properties (information provided by the sponsor)

Boiling point	106°C (760 mmHg)
Appearance at ordinary temperature	Colorless transparent liquid
Density	1.560 g/cm^3

f) Storage conditions

The test substance was put into a shaded and air-tight container and stored in 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

Olive oil including 3 w/v% of polyoxyethylene(20) sorbitan monooleate (Tween80)

b) Reason for selection

The test substance did not dissolve or was not suspended at a concentration of 20.0 w/v% to purified water, purified water including 1 w/v% of Tween80, purified water including 3 w/v% of Tween80, olive oil or olive oil including 1 w/v% of Tween80. However, the test substance was homogenously suspended to olive oil including 3 w/v% of Tween80 at a concentration of 20.0 w/v%. Additionally, the condition of the formulation such as

color did not change at room temperature four hours after the preparation. Therefore, olive oil including 3 w/v% of Tween80 was selected as a vehicle.

c) Manufacturer, grade, lot number and storage conditions

Name	Manufacturer	Grade	Lot number	Storage place	Storage temperature
Olive oil	Taisei Pharmaceutical Industries	Japanese Pharmacopoeia	605027	Reagent storage room	Room temperature
Tween80	Wako Pure Chemical Industries	CP	ECE6558	Reagent storage room	Cold place

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 we have the historical control data.

Five female rats at seven weeks old were obtained and quarantined/acclimatized for six days under group housing of three or fewer animals per cage. The animals were weighed at the receipt and six days after the receipt. Clinical signs were observed daily during the quarantine period. No abnormalities were found in the body weights or clinical signs 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 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 was assigned for the sighting study and four animals were assigned for the main study.

The animals were identified by painting using a red ink on the tail before the group allocation, and by painting on the hair using a red aqueous ink after the group allocation. Cages were identified by labels and racks were identified by indicating the study number, sex and dose levels.

The animals were eight weeks old at the administration of the sighting study and main study. Body weights were ranged 176.5 g at the administration of the sighting study and 174.1-181.6 g at that of main study. The individual body weights at the administration of the main study were confirmed to be within ±20% of that of the sighting study.

13.4 Animal husbandry

The animals were housed in the barrier-system animal rooms (quarantine room 1 and animal room 7) which were maintained at 21-25°C, relative humidity of 40-70%, 10-15 air changes per hour and photoperiod of 12 hour 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.

Undertrays 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 160915 and 161118, 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 at 3-5 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 result before the receipt of the animals was 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 the GHS hazard class of acute oral toxicity of the test substance was described as "Unclassified" in the safety data sheet provided from the sponsor, the dose level of the sighting study and main study were set at 2000 mg/kg (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)
Sighting	2000	10	20.0	1 (1)
Main	2000	10	20.0	4 (2-5)

14.3 Dosing formulation

Dosing formulation was prepared on each administration day. The test substance was weighed and Tween80 was added to the test substance and mixed. Then olive oil was added and mixed to suspend the test substance. The suspension was filled up to the prescribed volume with olive oil to prepare the dosing formulation. The dosing formulation was transferred into a plastic container and carried to the animal room.

The weight of the test substance and Tween80, and volume of the dosing formulations are shown below.

Study	Concentration of dosing formulation (w/v%)	Weight of test substance (g)	Weight of Tween80 (g)	Volume of formulation (mL)
Sighting	20.0	4.00107	0.60	20
Main	20.0	4.00000	0.60	20

14.4 Administration

The animals were fasted for 17-19 hours before the administration, and for 3-4 hours after the administration. The administration was performed at 10:05 for the sighting study and at 9:57-9:59 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. The dosing formulation was taken to the syringe while stirring with a magnetic stirrer.

14.5 Clinical observation

General clinical observation was conducted for the animals.

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.

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 animals were subjected to a gross necropsy 14 days after the administration. The 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.

14.8 Evaluation of the result

An acute oral toxicity of the test substance was classified according to GHS (Appendix 2).

15. DEVIATION FROM STUDY PLAN

No deviation from the study plan occurred.

16. TEST RESULTS

16.1 Clinical signs including mortality

At 2000 mg/kg, no mortalities or moribundities occurred and no abnormalities were observed in any animals.

16.2 Body weights

The results are shown in Table.

At 2000 mg/kg, no abnormalities were observed in any animals.

16.3 Macroscopic findings

At 2000 mg/kg, no abnormalities were observed in any animals.

17. DISCUSSION AND CONCLUSION

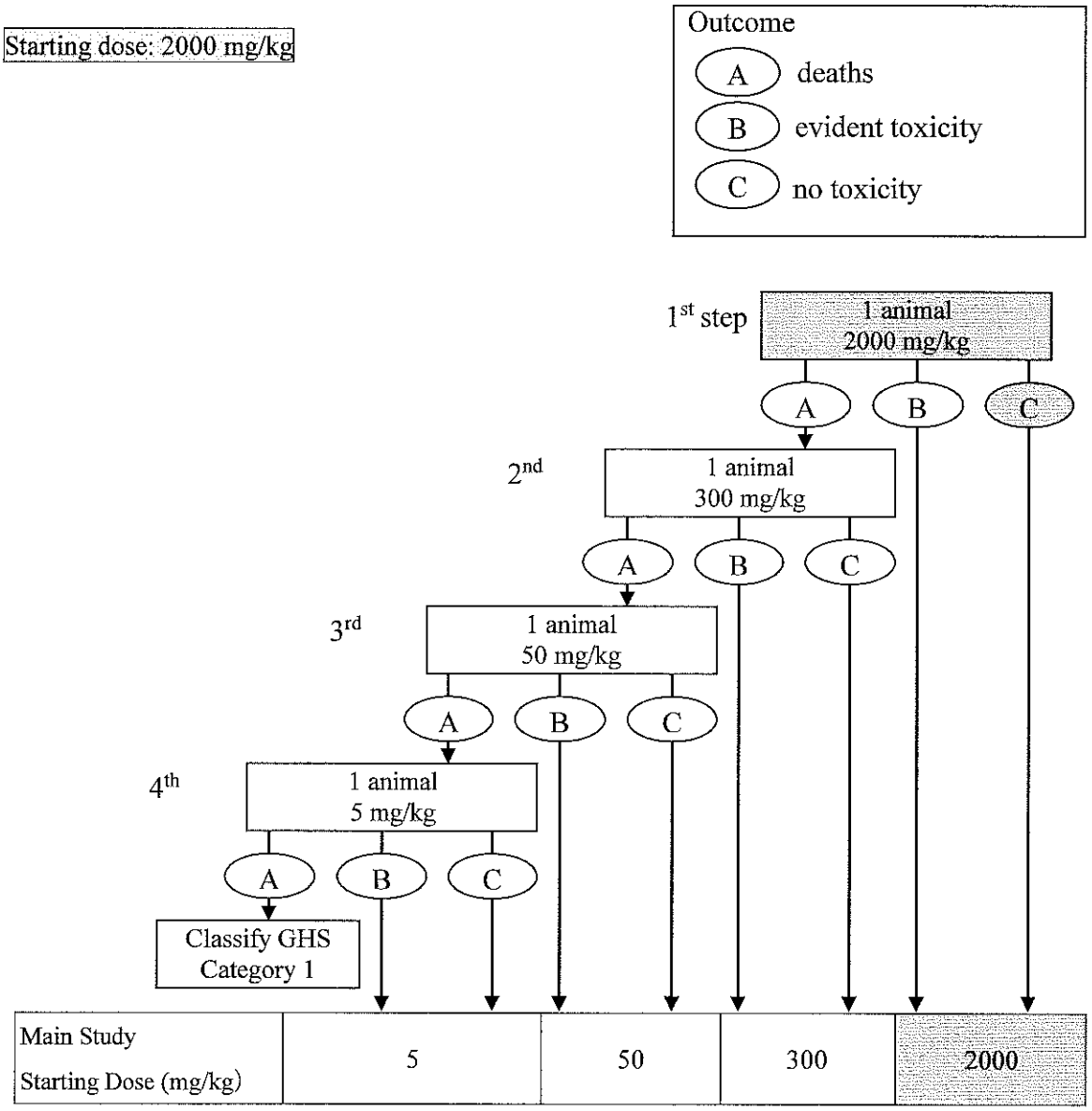
No mortalities or moribundities occurred at 2000 mg/kg and no abnormalities were observed in any animals in general clinical observation, body weight measurement or necropsy. Therefore, the hazard class of the acute oral toxicity of C6OLF in rats under the tested conditions was classified to "Category 5 or unclassified" of GHS.

Table Body weights

Study	Dose (mg/kg)	Animal No.	Body weights (g)			
			Day after administration			
			Initial	1 [a)]	7 [a)]	14 [a)]
Sighting study	2000	1	176.5	200.0 (23.5)	214.9 (14.9)	237.5 (22.6)
Main study	2000	2	181.6	200.5 (18.9)	224.2 (23.7)	239.3 (15.1)
		3	180.4	200.8 (20.4)	234.2 (33.4)	245.1 (10.9)
		4	174.1	188.9 (14.8)	205.8 (16.9)	212.2 (6.4)
		5	175.4	196.6 (21.2)	203.9 (7.3)	212.4 (8.5)

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

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

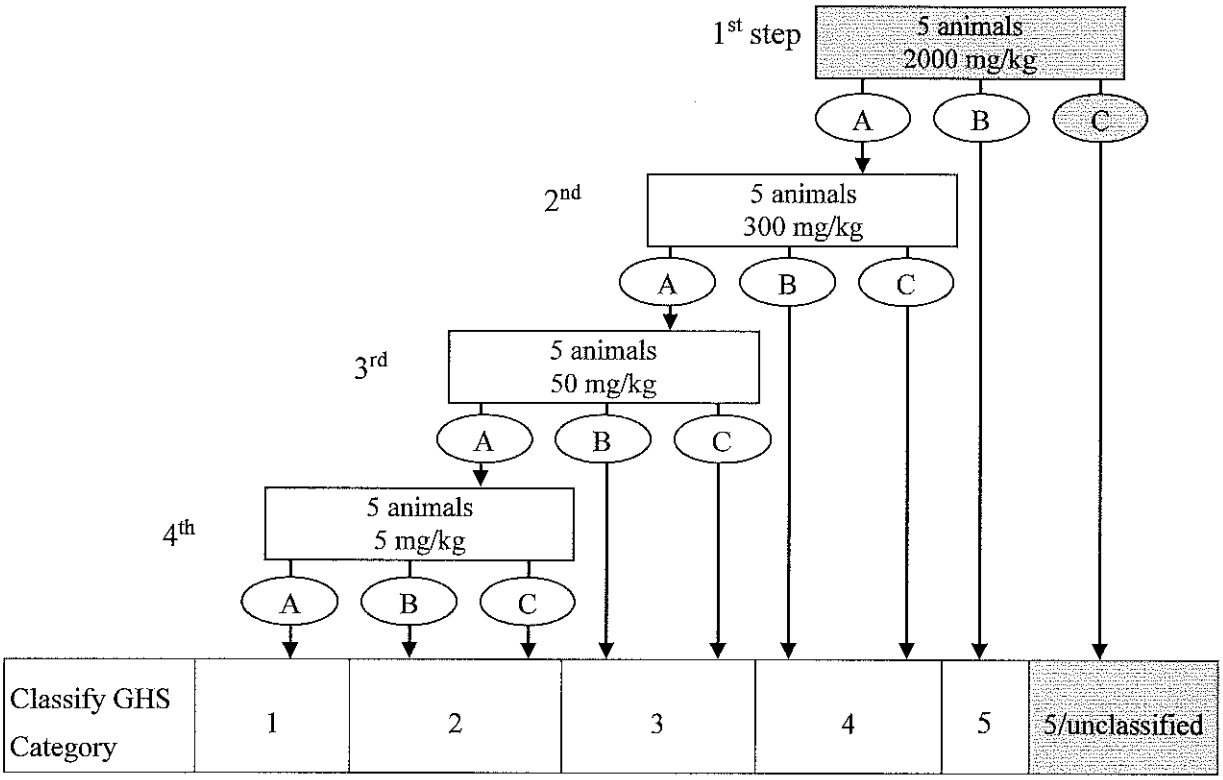


The colored cells show the procedure and the result of the sighting study.

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

Starting dose: 2000 mg/kg

Outcome
- A: ≥2 deaths
- B: ≥1 with evident toxicity and/or ≤ 1 death
- C: no toxicity



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

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

QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: Acute Oral Toxicity Study of C6OLF in Rats

Study Number: A16-0772

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	February 6, 2017	February 6, 2017
Study plan amendment No. 1	February 21, 2017	February 21, 2017
Animal management	February 21, 2017	February 21, 2017
Preparation of dosing formulations	February 21, 2017	February 21, 2017
Administration and clinical observations	February 21, 2017	February 21, 2017
Gross necropsy	March 7, 2017	March 8, 2017
Raw data and draft final report	March 21, 2017	March 21, 2017
Final report	March 22, 2017	March 22, 2017

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	February 7, 2017	March 22, 2017
Quarantine and acclimatization	February 7, 2017	March 22, 2017
Allocation and animal identification	December 26, 2016	March 22, 2017
Body weight measurement	February 7, 2017	March 22, 2017

Date: March 22, 2017

Quality Assurance Manager: ___