CERI

Receipt number	822-16-D-4116
Study number	A18-0069

FINAL REPORT

Acute Dermal Toxicity Study of C6OLF in Rats

April, 2017

Chemicals Evaluation and Research Institute, Japan, Hita

GLP STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor DAIKIN INDUSTRIES, LTD.

Title Acute Dermal Toxicity Study of C6OLF in Rats

Study number A18-0069

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: April 12, 2017
Date

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QUALITY ASSURANCE STATEMENT

1. TITLE

Acute Dermal 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, Oita 877-0061, Japan

4. OBJECTIVE

The objective of this study is to evaluate the acute dermal toxicity of C6OLF in rats originating from the single dermal application.

5. TEST METHOD

OECD Guidelines for the Testing of Chemicals, No. 402, Acute Dermal Toxicity, February 24, 1987

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 of CERI Hita" which referred to the following law, 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 20, 2017
Animal receipt	February 28, 2017
Application (experiment start)	March 7, 2017
Necropsy (experiment completion)	March 21, 2017
Study completion	April 12, 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, application, 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: April 12, 2017

Date

12. SUMMARY

The study was performed according to OECD Guideline for the Testing of Chemicals No. 402 to evaluate acute dermal toxicity of C6OLF.

The test substance was applied over the dorsal area of Crl:CD(SD) rats after hair of the animals was clipped. The applied area was covered with non-woven gauze and elastic adhesive bandage for 24 hours. The dose level was set at 2000 mg/kg which is the maximum dose in the test method. Five males at seven weeks old and five females at nine weeks old were used for the application. All animals were observed until 14 days after the application. Body weights were measured on days 0 (before the application), 7 and 14 after the application. The animals were subjected to gross necropsy at the end of the observation period.

No mortalities or moribundities occurred. No abnormalities associated with the application of the test substance were observed in the general clinical observation, body weights measurements or gross necropsy.

Since no mortalities or moribundities occurred at 2000 mg/kg, the LD50 value of C6OLF in rats under the test conditions was estimated to be more than 2000 mg/kg for males and females. The acute dermal toxicity hazard category was estimated to be "Category 5" or "Not classified" according to Globally Harmonized System of Classification and Labelling of Chemicals.

13. MATERIALS

13.1 Test substance

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

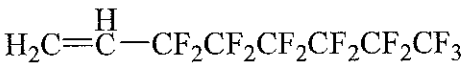
Chemical name	3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooct-1-ene
Other name	C6OLF
CAS number	25291-17-2

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



$$H_2C{=}\overset{H}{C}{-}CF_2CF_2CF_2CF_2CF_2CF_3$$

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 Animals

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

Six males at six weeks old and six female at eight weeks old were obtained and quarantined/acclimatized for six days under group housing of three 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.

The animals were allocated to groups using simple random sampling on six days after the receipt. After the allocation, the animals were housed individually. The clinical signs and excrement of the allocated animals were observed until the application day. The animals not allocated were excluded from the study after the allocation.

The animals were identified by painting using a red ink on the tail before the allocation, and by painting using a blue ink on the tail after the allocation. Cages were identified by labels and a rack was identified by indicating the study number, sex and dose levels.

The animals were seven weeks old for males and nine weeks old for females with body weights ranges of 232.2-255.7 g for males and 207.4-226.7 g for females at the application. The individual body weights at the application were confirmed to be within ±20% of the mean animal weight and also within a range of 200-300 g.

13.3 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). Undertrays were changed at the end of the quarantine/acclimation period and at the allocation, and changed twice a week after the allocation. Feeders, cages and racks were changed at the allocation.

The animals had free access to a pelleted diet (MF, lot number 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 dermal toxicity of the test substance was described as "Unclassified" in the safety data sheet provided from the sponsor, the dose level was set at 2000 mg/kg which is the maximum dose in the test method.

14.2 Dose level and number of animals etc.

Dose level (mg/kg)	Dosing volume (mL/kg)	Concentration of dosing formulation (%)	Number of animals (Animal number)	
			Male	Female
2000	1.28 a)	100	5 (1-5)	5 (6-10)

a): The value was calculated from the density (1.560 g/cm^3).

14.3 Dosing formulation

The test substance of 10.00 g was weighed into a brown glass bottle and carried to the animal room. The test substance was applied undiluted.

14.4 Application

The animals were dermally administered singly (for 24 hours). One day before the application, an area of approximately 5×10 cm on the back of the animals was clipped with a clipper (Matsushita Electric Works). The dosing formulation was applied to a non-woven gauze (5×5 cm, lot number 201509151, Ci Medical) with a syringe (TERUMO) at the volume of 1.28 mL/kg based on the body weight measured on the application day and the non-woven gauze was applied over the clipped dorsal area. The non-woven gauze was covered and fixed by elastic adhesive bandage (SILKYTEX5, lot number 51113254, ALCARE). The application was carried out from 10:11 to 10:21. Twenty four hours after the application, the non-woven gauze and elastic adhesive bandage were removed and residual test substance was removed using purified water (lot number PC160915, Takasugi Pharmaceutical) and absorbent cotton.

14.5 Clinical observation

General clinical observation was conducted for the animals.

The animals were observed continuously for 10 minutes after the application, and observed once 30 minutes and three hours after the application. The animals were observed once in the morning from 1 to 14 days after the application. On the next day of the application, the animals were observed after removal of the non-woven gauze and elastic adhesive bandage.

14.6 Measurement of body weight

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

14.7 Gross necropsy

The animals were subjected to a gross necropsy 14 days after the application. The animals were euthanized by bleeding from the abdominal aorta under isoflurane anesthesia, and application site, external surface of the body, all orifices, subcutis, cranial, thoracic, abdominal and pelvic cavities with their contents were observed.

14.8 Evaluation of result

Median lethal dose, LD50 value (mg/kg), is estimated according to the number of mortalities.

15. DEVIATION FROM STUDY PLAN

No deviation from the study plan occurred.

16. TEST RESULTS

16.1 Clinical signs including mortality

The results are shown in Table 1. No mortalities or moribundities occurred in any animals at 2000 mg/kg. Slight decreased spontaneous locomotion was sporadically observed in all animals of both sexes at 2000 mg/kg from five minutes to three hours after the application. This sign disappeared in all animals after removal of the non-woven gauze and elastic adhesive bandage on the next day of the application. Thereafter, no abnormalities were observed until 14 days after the application.

16.2 Body weights

The results are shown in Table 2. No abnormalities were observed in any animals.

16.3 Macroscopic findings

No abnormalities were observed in any animals.

17. DISCUSSION AND CONCLUSION

No mortalities or moribundities occurred in any animals although slight decreased spontaneous locomotion were observed in all animals of both sexes on the application day.

The decreased spontaneous locomotion has been observed in control groups of acute dermal toxic studies using elastic adhesive bandage to cover and fix the gauze in the testing facility and the sign disappeared after removal of the elastic adhesive bandage in this study. Therefore, the sign was considered to be caused by compression due to the elastic adhesive bandage and not to be associated with the test substance application.

Since no mortalities or moribundities occurred at 2000 mg/kg, the LD50 value of C6OLF in rats under the test conditions was estimated to be more than 2000 mg/kg for males and females.

TABLES

Table 1 Clinical signs

Dose (mg/kg)	Sex	Animal No.	Day after application																
			0				1	2	3	4	5	6	7	8	9	10	11	12	13	14
			(min.)			(hr.)														
			0[a)]-5	5-10	30	3														
2000	Male	1	-	DS*	-	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2	-	DS*	DS*	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		3	-	DS*	DS*	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		4	-	-	DS*	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		5	-	DS*	-	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Female	6	-	-	-	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		7	-	DS*	-	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		8	-	DS*	DS*	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		9	-	-	-	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		10	-	-	DS*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a): immediately after application.

-: no abnormalities detected.

DS: decreased spontaneous locomotion.

*: slight.

Table 2 Body weights

Dose (mg/kg)	Sex	Animal No.	Body weights (g)		
			Day after application		
			Initial	7 [a)]	14 [a)]
2000	Male	1	248.3	313.6 (65.3)	373.0 (59.4)
		2	246.3	310.9 (64.6)	367.9 (57.0)
		3	232.2	266.8 (34.6)	310.9 (44.1)
		4	255.7	318.2 (62.5)	361.8 (43.6)
		5	247.2	304.5 (57.3)	354.6 (50.1)
	Female	6	209.0	237.5 (28.5)	256.5 (19.0)
		7	214.3	239.4 (25.1)	252.2 (12.8)
		8	226.7	246.8 (20.1)	264.5 (17.7)
		9	207.4	233.6 (26.2)	244.9 (11.3)
		10	209.6	239.9 (30.3)	262.5 (22.6)

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

QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: Acute Dermal Toxicity Study of C6OLF in Rats

Study Number: A18-0069

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 20, 2017	February 20, 2017
Study plan amendment No. 1	February 22, 2017	February 22, 2017
Preparation of dosing formulations	March 7, 2017	March 8, 2017
Application and clinical observations	March 7, 2017	March 8, 2017
Gross necropsy	March 21, 2017	March 21, 2017
Raw data and draft final report	April 10, 2017	April 10, 2017
Draft final report No.2	April 10, 2017	April 10, 2017
Final report	April 12, 2017	April 12, 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	April 12, 2017
Quarantine and acclimatization	February 7, 2017	April 12, 2017
Animal management	February 15, 2017	April 12, 2017
Allocation and animal identification	December 26, 2016	April 12, 2017
Body weight measurement	February 7, 2017	April 12, 2017

Date: April 12, 2017

Quality Assurance Manager: _