CERI

Receipt number	822-17-D-4274
Study number	A18-0075

FINAL REPORT

Acute Dermal 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 Dermal Toxicity Study of APFHx in Rats

Study Number A18-0075

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

TABLE OF CONTENTS

1. TITLE

Acute Dermal 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 dermal toxicity of APFHx in rats originating from the dermal application of a single dose.

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 in the testing facility which refer 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
Application (experiment start)	April 3, 2018
Necropsy (experiment completion)	April 17, 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, 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: May 9, 2018

Date

12. SUMMARY

The study was performed according to OECD Guidelines for the Testing of Chemicals, No. 402 to evaluate the acute dermal toxicity of APFHx.

The test substance was dissolved in purified water and 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 limited dose in the test method. Five males at seven weeks old and five females at nine weeks old were used for the application. Clinical signs were observed daily for 14 days and body weights were measured 0 (before administration), 7 and 14 days after the administration. The animals were subjected to a gross necropsy 14 days after the administration.

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 dermal LD50 value of APFHx in rats under the tested conditions was considered to be more than 2000 mg/kg for males and females. The hazard class of the acute dermal toxicity of APFHx 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) 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

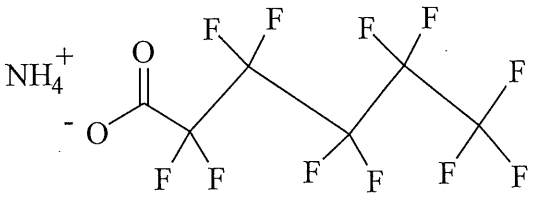
b) Supplier and lot number (information provided by the sponsor)

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.

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 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 allocated to groups using simple random sampling six days after the receipt. After the allocation, the animals were housed individually. The animals not allocated were excluded from the study after the allocation.

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

The animals were seven weeks old for males and nine weeks old for females with body weights ranges of 250.4 g to 254.4 g for males and 218.5 g to 232.5 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 g to 300 g.

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

The dose level was set at 2000 mg/kg based on a request of a limit test from the sponsor.

14.2 Dose level and number of animals etc.

Dose level (mg/kg)	Dose volume (mL/kg)	Concentration of dosing formulation (w/v%)	Number of animals (Animal number)	
			Male	Female
2000	10	20.0	5 (1-5)	5 (6-10)

14.3 Dosing formulation

Dosing formulation was prepared on the application day. The test substance of 10.00 g was weighed and mixed with purified water to be dissolved. The solution was filled up to 50 mL with purified water to prepare the 20.0 w/v% of dosing formulation. The dosing formulation was transferred into a plastic container and carried to the animal room. The dosing formulation was applied within two hours after the preparation.

14.4 Application

The animals were dermally administered in a single dose (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 homogeneously applied to a non-woven gauze (5×5 cm, lot number 201509151, Ci Medical) with a syringe (TERUMO) at the volume of 10 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 10:23 to 10:33. 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 PC171219, Takasugi Pharmaceutical) and absorbent cotton.

14.5 General clinical observation

The clinical signs including the mortalities were observed.

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 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. 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 and moribundities.

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.

At 2000 mg/kg, no mortalities or moribundities occurred in any animals. Slight decreased spontaneous locomotion was observed in all animals of males and females between just after the application and three hours after the application. This sign disappeared in all animals one day after 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

The results are shown in Table 3.

Pelvic dilatation of the right kidney was observed in one male. No abnormalities were observed in any other animals.

17. DISCUSSION AND CONCLUSION

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

Slight 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. In this study, the sign appeared from just after the application and no other abnormalities were observed. 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.

The pelvic dilatation observed in one male was considered as a spontaneous lesion, because it was unilateral change and pelvic dilatation has been observed in the animals which are not dosed with test substance.

Since no mortalities or moribundities occurred at 2000 mg/kg, the dermal LD50 value of APFHx in rats under the tested conditions was considered to be more than 2000 mg/kg for males and females. The hazard class of the acute dermal toxicity of APFHx in rats under the tested conditions was classified to "Category 5 or unclassified" of Globally Harmonized System of Classification and Labelling of Chemicals.

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	DSL*	DSL*	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2	DSL*	DSL*	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		3	DSL*	DSL*	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		4	DSL*	DSL*	DSL*	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		5	DSL*	DSL*	DSL*	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Female	6	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		7	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		8	DSL*	-	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		9	DSL*	DSL*	DSL*	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		10	DSL*	DSL*	-	DSL*	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a): immediately after application.

-: no abnormalities detected.

DSL: 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	252.3	312.0 (59.7)	350.6 (38.6)
		2	252.9	307.5 (54.6)	336.7 (29.2)
		3	254.4	319.8 (65.4)	378.2 (58.4)
		4	254.0	312.3 (58.3)	361.7 (49.4)
		5	250.4	308.1 (57.7)	353.9 (45.8)
	Female	6	225.4	248.2 (22.8)	253.7 (5.5)
		7	225.7	245.5 (19.8)	263.3 (17.8)
		8	218.5	245.6 (27.1)	258.9 (13.3)
		9	232.5	259.2 (26.7)	272.7 (13.5)
		10	219.9	257.9 (38.0)	280.8 (22.9)

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

Table 3 Macroscopic findings

Dose (mg/kg)	Sex	Animal No.	Fate	Macroscopic findings
2000	Male	1	ss	No abnormalities detected
		2	ss	No abnormalities detected
		3	ss	No abnormalities detected
		4	ss	No abnormalities detected
		5	ss	Kidney Pelvic dilatation (right)
	Female	6	ss	No abnormalities detected
		7	ss	No abnormalities detected
		8	ss	No abnormalities detected
		9	ss	No abnormalities detected
		10	ss	No abnormalities detected

ss: scheduled sacrifice animal.

QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: Acute Dermal Toxicity Study of APFHx in Rats

Study Number: A18-0075

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
Application and clinical observations	April 3, 2018	April 3, 2018
Gross necropsy	April 17, 2018	April 17, 2018
Raw data and draft final report	May 2, 2018	May 7, 2018
Draft final report No. 2	May 2, 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
Allocation and animal identification	January 15, 2018	May 9, 2018
Body weight measurement	March 9, 2018	May 9, 2018

Date: May 9, 2018

Quality Assurance Manager: ___