B060991

Final Report

A Preliminary Seven-Day Repeated Dose Oral Toxicity Study of APFHx in Rats

(Study No. B060991)

Date: October 25, 2006

Mitsubishi Chemical Safety Institute Ltd.

1. Table of Contents

2. Study Outline

2.1 Title

A Preliminary Seven-Day Repeated Dose Oral Toxicity Study of APFHx in Rats

2.2 Study number

B060991

2.3 Purpose

This study was conducted to measure the liver weight after a 7-day repeated oral administration of APFHx to rats, and the results were referred to in the dose findings for the toxicogenomics study.

2.4 Guideline

Not applicable

2.5 GLP

Not applicable

2.6 Sponsor

Daikin Industries, Ltd.
1-1, Nishi Hitotsuya, Settsu-shi, Osaka, Japan

2.7 Organization under contract

Mitsubishi Chemical Safety Institute Ltd.
1-30, Shiba 2-chome, Minato-ku, Tokyo, Japan

2.8 Test facility

Kashima Laboratory, Mitsubishi Chemical Safety Institute Ltd.
14, Sunayama, Kamisu-shi, Ibaraki, Japan

2.9 Study director

Kotaro Yamashita
Toxicology Division I, Kashima Laboratory
Mitsubishi Chemical Safety Institute Ltd.

2.10 Study contributors

Receipt, quarantine and acclimation of animals, grouping, and identification of animals: Kaori Nose, Yoko Kuroda, and Takeshi Kawasuso

Preparation of the dosing solutions:

Hideko Nakamura

Administration:
Kaori Nose, Yoko Kuroda, and Takeshi Kawasuso

Clinical observation and body weight measurement:
Kaori Nose, Yoko Kuroda, and Takeshi Kawasuso

Measurement of organ weight and necropsy:
Kaori Nose, Yoko Kuroda, Takeshi Kawasuso, and Minoru Tsuchitani

2.11 Schedule

Initiation of the study	July 4, 2006
Receipt of animals	July 5, 2006
Start of administration	July 12, 2006
Necropsy	July 19, 2006
Completion of the study	October 25, 2006

2.12 Retention

The items in the next section are retained in the archives of the Kashima Laboratory, Mitsubishi Chemical Safety Institute Ltd. These will be retained for 5 years after the submission of the final report of the toxicogenomics study (study No. B060900), after which time, the sponsor will be contacted to determine the disposition of these items.

2.13 Retention materials

(1) Protocol
(2) Test substance records
(3) Test animal records
(4) Study result records
(5) Documentation
(6) Final report

3. Study Director Signature

Sponsor: Daikin Industries, Ltd.

Title: A Preliminary Seven-Day Repeated Dose Oral Toxicity Study of APFHx in Rats

Study No.: B060991

Study Director: Signed and sealed Date: October 25, 2006

Toxicology Division I, Kashima Laboratory
Mitsubishi Chemical Safety Institute Ltd.

4. Summary

APFHx was administered repeatedly by oral gavage at doses of 0, 1.5, 15, and 150 mg/kg/day (as perfluorohexanoic acid ammonium salt) to F344/DuCrlCrlj rats (3 males per group) for 7 days to examine its influence.

There were no changes considered to be test substance-related in the clinical observation, body weight measurement, organ weight measurement (liver weight), or necropsy.

In conclusion, no obvious APFHx-related changes were noted under the conditions employed in this study.

5. Materials and Methods

5.1 Test substance

5.1.1 Name

APFHx (CAS 21615-47-4: ammonium undecafluorohexanoate)

5.1.2 Chemical name

Perfluorohexanoic acid ammonium salt (47.5 mass% solution)
Dose levels and concentrations of the dosing solutions are expressed as perfluorohexanoic acid ammonium salt (conversion factor: 2.11).

5.1.3 Supplier

Daikin Industries, Ltd.

5.1.4 Lot No.

060427

5.1.5 Storage conditions

Room temperature (acceptable range: 10 to 30°C, actual range: 18.5 to 24.6°C).

5.2 Vehicle

5.2.1 Name

Purified water (water for injection, Otsuka Pharmaceutical Factory, Inc., Lot No. 6C98)

5.3 Experimental animals

5.3.1 Species

Rat

5.3.2 Strain

F344/DuCrlCrlj

5.3.3 Reason for the choice of the strain

This strain is used for the planned toxicogenomics study.

5.3.4 Microbiological level

SPF

5.3.5 Supplier

Charles River Laboratories Japan, Inc.

5.3.6 Number of animals purchased
18 males

5.3.7 Quarantine and acclimation
Animals were quarantined for 5 days after arrival. They were subjected to clinical observation once a day and confirmed to be in good health. The body weights were measured on the day of receipt and on the final day of quarantine and confirmed normal body weight gains. Acclimation was further continued and all animals were subjected to clinical observation once a day. It was confirmed that all animals were in good health.

5.3.8 Age at the start of administration
5 weeks old

5.3.9 Body weight at the start of administration
Body weights of animals used in the study ranged from 98 to 110 g. It was confirmed that the body weights of all animals were within ±20% of the mean body weight.

5.3.10 Grouping
The animals were assigned to each group by stratified-by-weight randomization method on the day before the start of dosing to give homogeneous distribution of the body weight among the groups.

5.3.11 Identification
Animals were identified by oil-based ink marks on their tails, and the cages were identified by labels listing the following information: before grouping; study number, cage number, quarantine/acclimation animal numbers, animal species, strain, and sex, after the grouping; study number, group name (dose level), animal numbers, animal species, strain, and sex.

5.3.12 Disposition of animals unused
The animals unused in the study were excluded from the study on the day after the start of dosing. These animals were euthanized.

5.4 Animal management
5.4.1 Animal room
Rat/Mouse room (No. 6126)

5.4.2 Environmental conditions

5.4.2.1 Temperature

Actual range: 21.7 to 22.8°C, acceptable range: 19.0 to 25.0°C

5.4.2.2 Relative humidity

Actual range: 51.4% to 59.9%, acceptable range: 35.0% to 75.0%

5.4.2.3 Ventilation

About 10 to 30 times/hour (fresh, filtered air)

5.4.2.4 Lighting

12 hours per day (7:00 to 19:00)

5.4.3 Facility equipment

5.4.3.1 Cages

Autoclaved polycarbonate cages (265W × 426D × 200H mm, Tokiwa Kagaku Kikai Co., Ltd.) and autoclaved stainless steel lids as feeders (Tokiwa Kagaku Kikai Co., Ltd.) were used and replaced on the day of grouping.

5.4.3.2 Watering bottles

Autoclaved polycarbonate watering bottles (500 mL, Tokiwa Kagaku Kikai Co., Ltd.) were used and replaced at the same time as the cages.

5.4.3.3 Racks

Autoclaved stainless steel racks with a polyvinyl chloride sliding door (front-to-back airflow ventilation, Toyoriko Co., Ltd.) were used. The door was disinfected by formalin.

5.4.4 Bedding

5.4.4.1 Description

Autoclaved hardwood chips (Beta-Chip, Charles River Laboratories Japan, Inc.) were used and replaced at the same time as the cages.

5.4.4.2 Confirmation of contaminants

Analysis data by Japan Food Research Laboratories were periodically obtained from the manufacturer. It was confirmed that the levels of the environmental contaminants, such as residual pesticides in the bedding met the standards of our Standard Operating Procedure (SOP).

5.4.5 Diet
5.4.5.1 Description
Autoclaved pellet diet for experimental animals (CRF-1, Oriental Yeast Co., Ltd.)

5.4.5.2 Supply method
Animals were allowed free access to the diet. The diet was replaced at the same time as the cages.

5.4.5.3 Confirmation of contaminants
Analysis data by Japan Food Research Laboratories, to which the supplier contracted the works, were obtained from the supplier. It was confirmed that the levels of the environmental contaminants, such as residual pesticides in the lot used in this study met the standards of our SOP.

5.4.6 Drinking water
5.4.6.1 Description
Tap water, irradiated by UV rays after passing through a 5-μm filter

5.4.6.2 Supply method
Animals were allowed free access to drinking water. Water was replaced at the same time as the watering bottles.

5.4.6.3 Analysis
The tap water was periodically analyzed by Dia Analysis Service Inc. twice a year. From the analytical data, it was confirmed that the quality of the water met the standards of our SOP.

5.4.7 Number of animals per cage
One animal per cage

5.5 Administration
5.5.1 Route/Method
Oral (by gavage)
The test substance was administered orally using a disposable syringe attached to a gastric tube for rats.

5.5.2 Frequency/Duration
Once a day in the morning for 7 days

5.5.3 Dose
Dose levels were set at 1.5, 15, and 150 mg/kg/day. The control group (0 mg/kg/day) treated with the vehicle (purified water) was also set.

5.5.4 Dose volume
The dose volume was set at 10 mL/kg. The individual volume was calculated on the basis of the most recently recorded body weight.

5.6 Dosing solutions
5.6.1 Frequency
Once on the day before the start of dosing

5.6.2 Preparation method
Dosing solutions were prepared under non-UV fluorescent light. The test substance (1.58253 g) was weighed and added with vehicle (purified water) up to 50 mL to make the dosing solution for the high dose group (15 mg/mL as perfluorohexanoic acid ammonium salt). The 15 mg/mL solution (5 mL) was collected and added with vehicle up to 50 mL to make the dosing solution for the middle dose group (1.5 mg/mL). The 1.5 mg/mL solution (5 mL) was collected and added with vehicle up to 50 mL to make the dosing solution for the low dose group (0.15 mg/mL). The dosing solutions were divided into brown glass bottles for each dosing day and stored in a refrigerator (actual range: 1.9 to 6.1°C, acceptable range: 1 to 10°C) under light-resistant conditions for no longer than 7 days after preparation until the day of the final dosing.

5.7 Study design

Group	Concentration (mg/mL)	Number of animals (animal number)
Control *1	0	3 males (10101 to 10103)
1.5 mg/kg/day	0.15	3 males (10201 to 10203)
15 mg/kg/day	1.5	3 males (10301 to 10303)
150 mg/kg/day	15	3 males (10401 to 10403)

*1: Treated with the vehicle (purified water) alone
The dose levels and concentrations of the dosing solutions are expressed as perfluorohexanoic acid ammonium salt.

5.8 Observation and measurements

The following parameters were examined. The first day of dosing was designated as Day 1.

5.8.1 Clinical observation

Animals were observed twice a day (before dosing and about 30 minutes after dosing) during the dosing period, and once in the morning on the necropsy day.

5.8.2 Body weight

All animals were weighed on Days 1, 4, and 8. An electronic balance (PB3002-S; Mettler-Toledo K.K.) was used for weighing.

5.8.3 Pathological examinations

5.8.3.1 Organ weight

The livers from all animals were weighed at necropsy. An electronic balance (AG204; Mettler-Toledo K.K.) was used for weighing. The body weights were measured on the day of necropsy and used to calculate the relative organ weights.

5.8.3.2 Necropsy

All animals were subjected to necropsy under non-fasting conditions after the end of the clinical observation on Day 8. All animals were euthanized by exsanguination via the abdominal aorta under intraperitoneal pentobarbital sodium anesthesia (Nembutal, Dainippon Pharmaceutical Co., Ltd.) and then subjected to necropsy.

5.9 Statistical analysis

Data of the body weight and absolute and relative weights of the liver were analyzed by multiple comparison tests for the statistical significance. In the analysis, the homogeneity of the variance among the groups was first tested by Bartlett's test. When the variance was demonstrated to be homogeneous, one-way analysis of variance was applied. When the variance was heterogeneous, Kruskal-Wallis test was applied. When a significant difference was detected among the groups, Dunnett's test (when the variance was homogeneous) or Steel test (when the variance was heterogeneous) was performed to compare the mean in the control group with that in each dose group.
Bartlett's test, one-way analysis of variance, and Kruskal-Wallis test were conducted at the significance level of 5%, and the other tests were conducted at the significance levels of 5% and 1%. MiTOX® (Mitsui Zosen Systems Research Inc.) was used for statistical analysis. The analysis was not performed on the results of the clinical observation or necropsy.

5.10 Computer system

Toxicological Data Processing System (MiTOX®, Mitsui Zosen Systems Research Inc.) was used to collect and analyze the data indicated below. The scope and schedule of data collection, etc., were registered for the applicable computer system protocol. B060991_ (_ indicates a blank space) was used as the computer system protocol number.

Used items: Body weight measurement, grouping, calculation of dosing volume, clinical observation, organ weight, necropsy

6. Results

6.1 Clinical signs

Clinical signs are shown in Table 1.
No abnormalities were noted in any animal during the experimental period.

6.2 Body weight

Body weight data are shown in Table 2.
Body weight increased normally in all animals during the experimental period.

6.3 Organ weight

The data of absolute and relative weights of the liver are shown in Tables 3 and 4.
There were no test substance-related changes at any dose level.

6.4 Necropsy findings

Necropsy findings are shown in Table 5.
No abnormalities were noted in any animal.

7. Discussion and Conclusion

APFHx was administered repeatedly by oral gavage at doses of 0, 1.5, 15, and 150 mg/kg/day (as perfluorohexanoic acid ammonium salt) to F344/DuCrlCrlj rats for 7 days to examine its influence.

There were no changes considered to be test substance-related in the clinical observation, body weight measurement, organ weight measurement (liver weight), or necropsy.

In conclusion, no obvious APFHx-related changes were noted under the conditions employed in this study.

Table 1 Clinical Sign - Summary Male

Test Substance		Day	1		2		3		4		5		6		7		8
Dose	Findings	Time	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10
APFHx	Number of Animals		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
0 mg/kg	No Abnormality		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
APFHx	Number of Animals		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
1.5 mg/kg	No Abnormality		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
APFHx	Number of Animals		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
15 mg/kg	No Abnormality		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
APFHx	Number of Animals		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
150 mg/kg	No Abnormality		3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Time 10 , before dosing; Time 20 , about 30 min after dosing

Table 2 Body Weight Male

Unit : g

Test Substance Dose	Animal Number	Day 1	4	8
APFHx 0 mg/kg	10101	106	117	129
	10102	107	120	134
	10103	109	121	139
	Mean	107.3	119.3	134.0
	S.D.	1.5	2.1	5.0
	n	3	3	3
APFHx 1.5 mg/kg	10201	110	121	136
	10202	106	120	135
	10203	102	115	129
	Mean	106.0	118.7	133.3
	S.D.	4.0	3.2	3.8
	n	3	3	3
APFHx 15 mg/kg	10301	98	109	119
	10302	106	118	133
	10303	109	124	145
	Mean	104.3	117.0	132.3
	S.D.	5.7	7.5	13.0
	n	3	3	3
APFHx 150 mg/kg	10401	105	118	135
	10402	109	120	136
	10403	103	116	136
	Mean	105.7	118.0	135.7
	S.D.	3.1	2.0	0.6
	n	3	3	3

Table 3 Organ Weight Male

Test Substance Dose	Animal Number	Final Body Weight g	Liver g
APFHx 0 mg/kg	10101	129	5.59
	10102	134	5.80
	10103	139	5.87
	Mean	134.0	5.753
	S.D.	5.0	0.146
	n	3	3
APFHx 1.5 mg/kg	10201	136	5.62
	10202	135	5.70
	10203	129	5.44
	Mean	133.3	5.587
	S.D.	3.8	0.133
	n	3	3
APFHx 15 mg/kg	10301	119	5.02
	10302	133	5.51
	10303	145	6.15
	Mean	132.3	5.560
	S.D.	13.0	0.567
	n	3	3
APFHx 150 mg/kg	10401	135	5.79
	10402	136	6.22
	10403	136	6.07
	Mean	135.7	6.027
	S.D.	0.6	0.218
	n	3	3

Table 4 Relative Organ Weight Male

Test Substance Dose	Animal Number	Final Body Weight g	Liver %
APFHx	10101	129	4.33
0 mg/kg	10102	134	4.33
	10103	139	4.22
	Mean	134.0	4.293
	S.D.	5.0	0.064
	n	3	3
APFHx	10201	136	4.13
1.5 mg/kg	10202	135	4.22
	10203	129	4.22
	Mean	133.3	4.190
	S.D.	3.8	0.052
	n	3	3
APFHx	10301	119	4.22
15 mg/kg	10302	133	4.14
	10303	145	4.24
	Mean	132.3	4.200
	S.D.	13.0	0.053
	n	3	3
APFHx	10401	135	4.29
150 mg/kg	10402	136	4.57
	10403	136	4.46
	Mean	135.7	4.440
	S.D.	0.6	0.141
	n	3	3

Table 5 Necropsy Findings Scheduled Sacrifice (Day 8)

	Sex	Male											
	Test Substance	APFHx			APFHx			APFHx			APFHx		
	Dose	0			1.5			15			150		
	Dose Unit	mg/kg			mg/kg			mg/kg			mg/kg		
	Animal No.	1	1	1	1	1	1	1	1	1	1	1	1
		0	0	0	0	0	0	0	0	0	0	0	0
		1	1	1	2	2	2	3	3	3	4	4	4
		0	0	0	0	0	0	0	0	0	0	0	0
		1	2	3	1	2	3	1	2	3	1	2	3
Organ Findings	Abnormality	N	N	N	N	N	N	N	N	N	N	N	N
No abnormalities													

N , Finding absent

Appendix 1-1 Clinical Sign APFHx 0 mg/kg Male

Animal		Day	1		2		3		4		5		6		7		8
Number	Findings	Time	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10
10101	No Abnormality																
10102	No Abnormality																
10103	No Abnormality																

Time 10 , before dosing; Time 20 , about 30 min after dosing

Appendix 1- 2 Clinical Sign APFHx 1.5 mg/kg Male

Animal		Day	1		2		3		4		5		6		7		8
Number	Findings	Time	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10
10201	No Abnormality																
10202	No Abnormality																
10203	No Abnormality																

Time 10 , before dosing; Time 20 , about 30 min after dosing

Appendix 1- 3 Clinical Sign APFHx 15 mg/kg Male

Animal		Day	1		2		3		4		5		6		7		8
Number	Findings	Time	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10
10301	No Abnormality																
10302	No Abnormality																
10303	No Abnormality																

Time 10 , before dosing; Time 20 , about 30 min after dosing

Appendix 1- 4 Clinical Sign APFHx 150 mg/kg Male

Animal		Day	1		2		3		4		5		6		7		8
Number	Findings	Time	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10
10401	No Abnormality																
10402	No Abnormality																
10403	No Abnormality																

Time 10 , before dosing; Time 20 , about 30 min after dosing

Translation Statement

Sponsor: Daikin Industries, Ltd.

Title: A Preliminary Seven-Day Repeated Dose Oral Toxicity Study of APFHx in Rats

Study number: B060991

The original final report of this study was written in Japanese. I, hereby, declare that the original final report was faithfully translated into English as accurately as possible.

Translator: Date: January 12, 2017

Safety Assessment Department, Nonclinical Research Center,
Drug Development Service Segment, LSI Medience Corporation