

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.



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## **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

B060991

**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  
Kotaro Yamashita  
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/DuCrIcrlj 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/DuCrjCrj

**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  $\pm 20\%$  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- $\mu$ m 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 <sup>*1</sup>	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<sup>®</sup> (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<sup>®</sup>, 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/DuCrI CrIj 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 Dose	Male																							
	Day 1		2		3		4		5		6		7		8									
Findings	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10	20								
APFHx 0 mg/kg	3 3		3 3		3 3		3 3		3 3		3 3		3 3		3 3		3 3							
Number of Animals	3		3		3		3		3		3		3		3		3							
No Abnormality	3		3		3		3		3		3		3		3		3							
APFHx 1.5 mg/kg	3 3		3 3		3 3		3 3		3 3		3 3		3 3		3 3		3 3							
Number of Animals	3		3		3		3		3		3		3		3		3							
No Abnormality	3		3		3		3		3		3		3		3		3							
APFHx 15 mg/kg	3 3		3 3		3 3		3 3		3 3		3 3		3 3		3 3		3 3							
Number of Animals	3		3		3		3		3		3		3		3		3							
No Abnormality	3		3		3		3		3		3		3		3		3							
APFHx 150 mg/kg	3 3		3 3		3 3		3 3		3 3		3 3		3 3		3 3		3 3							
Number of Animals	3		3		3		3		3		3		3		3		3							
No Abnormality	3		3		3		3		3		3		3		3		3							

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



Test Substance Dose	Animal Number	Day	Male				Unit : g
			1	4	8	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	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	Final Body Weight		Liver %
	Animal Number	g	
APFHx 0 mg/kg	10101	129	4.33
	10102	134	4.33
	10103	139	4.22
	Mean	134.0	4.293
S.D.	5.0	0.064	
n	3	3	
APFHx 1.5 mg/kg	10201	136	4.13
	10202	135	4.22
	10203	129	4.22
	Mean	133.3	4.190
S.D.	3.8	0.052	
n	3	3	
APFHx 15 mg/kg	10301	119	4.22
	10302	133	4.14
	10303	145	4.24
	Mean	132.3	4.200
S.D.	13.0	0.053	
n	3	3	
APFHx 150 mg/kg	10401	135	4.29
	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	Test Substance	Dose	Dose Unit	Animal No.	Male		APFHx		APFHx		APFHx	
					APFHx	mg/kg	1.5	mg/kg	15	mg/kg	150	mg/kg
0												
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	1	2	2	2	3	3	4	4	4
0		0	0	0	0	0	0	0	0	0	0	0
1		1	2	3	1	2	3	1	2	3	1	2
	Abnormality				N	N	N	N	N	N	N	N

Organ Findings

No abnormalities

N, Finding absent

Appendix 1-1	Clinical Sign	APFHx	0 mg/kg	Male					
Animal Number	Day	1	2	3	4	5	6	7	8
Findings	Time	10	20	10	20	10	20	10	20
10101									
10102									
10103									

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

Appendix 1-2	Clinical Sign	APFHx	1.5 mg/kg	Male					
Animal Number	Day	1	2	3	4	5	6	7	8
Findings	Time	10	20	10	20	10	20	10	20
10201									
10202									
10203									

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

Appendix 1-3	Clinical Sign	APPHx	15 mg/kg	Male					
Animal Number	Day	1	2	3	4	5	6	7	8
Findings	Time	10	20	10	20	10	20	10	20
10301									
10302									
10303									

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	4
Number	Time	10	10	10
10401	No Abnormality	20	20	20
10402	No Abnormality	10	10	10
10403	No Abnormality	20	20	20

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: Kotaro Yamashita Date: January 12, 2017  
Kotaro Yamashita  
Safety Assessment Department, Nonclinical Research Center,  
Drug Development Service Segment, LSI Medience Corporation