CERI

Receipt Number	832-17-T-9323
Study Number	K10-0379

FINAL REPORT

In vitro Skin Corrosion Test of APFHx Using EpiDerm™ SCT (EPI-200)

May, 2018

Chemicals Evaluation and Research Institute, Japan, Hita

This document is exact copy of the original.

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GLP STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: *In vitro* Skin Corrosion Test of APFHx Using EpiDerm™ SCT (EPI-200)

Study Number: K10-0379

The study described in this report was conducted in compliance with the following GLP principle.

OECD Principles of Good Laboratory Practice, November 26, 1997

I also confirmed that this report accurately reflected the raw data and the test data were valid.

Study Director: _____ May 11, 2018
Date

TABLE OF CONTENTS

1. TITLE

In vitro Skin Corrosion Test of APFHx Using EpiDerm™ SCT (EPI-200)

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 ability of the test substance to induce skin corrosion is investigated using EpiDerm™ SCT (EPI-200).

5. TEST METHOD

"OECD Guidelines for the Testing of Chemicals, No. 431, *In vitro* skin corrosion: reconstructed human epidermis (RHE) test method" (Adopted: July 29, 2016)

6. GLP PRINCIPLE

OECD Principles of Good Laboratory Practice, November 26, 1997

7. DATES

Study Initiation Date	March 22, 2018
Experiment Starting Date	March 28, 2018
Experiment Completion Date	March 28, 2018
Study Completion Date	May 11, 2018

8. PERSONNEL CONCERNED WITH STUDY

Study Director:

Study Staff:

(Exposure of test substance, rinse of tissue and measurement of optical density (OD))

9. STORAGE AND RETENTION PERIOD OF DATA

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 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.

10. APPROVAL BY AUTHOR

Study Director:

May 11, 2018
Date

11. SUMMARY

The ability of APFHx to induce skin corrosion was investigated using EpiDerm™ SCT (EPI-200).

As a result of the skin corrosion test, the cell viabilities treated by APFHx in the 3-minute and 60-minute exposures were 94.4% and 5.0%, respectively.

Consequently, it was concluded that APFHx was "Corrosive" (UN GHS Category 1B and 1C) under the present test conditions.

12. MATERIALS

12.1 Test Substance and Control Substances

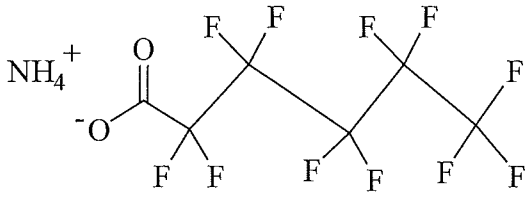
a) Test substance (information provided by the sponsor)

1) Chemical name, etc.

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

2) Structural formula, etc.

Structural formula



Molecular formula	$C_6H_4F_{11}NO_2$
Molecular weight	331.08

3) Purity, etc.

Purity	99.8%
Impurity	Water: 0.2%
Supplier	DAIKIN INDUSTRIES, LTD.
Lot number	C150S1703

4) Physicochemical properties

Appearance at ordinary temperature	White powder
Stability	Stable in storage condition

5) Storage conditions

The test substance was put into a light shielding and airtight container and stored in a desiccator at room temperature in the test substance storage room (permissible range: from 10°C to 30°C).

6) Safety

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.

b) Negative control substance

1) Name

Distilled water

2) Manufacturer, lot number and grade

Manufacturer	Otsuka Pharmaceutical Factory
Lot number	K7F81
Grade	for injection

3) Reason for selection
Distilled water is recommended in the test method.
4) Storage conditions
Distilled water was stored at room temperature in the preparation room No. 2.

c) Positive control substance
1) Name
8N Potassium hydroxide solution
2) Preparation method and storage conditions
Potassium hydroxide (Lot number: LKJ3109, special grade, Wako Pure Chemical Industries) was dissolved in distilled water (Lot number: K7F81, for injection, Otsuka Pharmaceutical Factory).
3) Reason for selection
8N Potassium hydroxide solution is recommended in the test method.
4) Storage conditions
Potassium hydroxide was stored at room temperature in the test substance storage room (permissible range: from 10°C to 30°C). 8N Potassium hydroxide solution was prepared just before use and stored at room temperature in the cell experimental room No. 1.

12.2 Test Kit
a) Name
EPI-200 SCT kit
b) Manufacturer
MatTek Corporation
c) Receipt date
March 27, 2018
d) Components
EpiDerm tissue (tissue insert, Lot number: 28302, manufactured on March 22, 2018)
Assay medium (medium, Lot number: 032118CMHA)
Phosphate buffered saline without Mg^{2+} and Ca^{2+} (Lot number: 022818APEA)
Nylon mesh (Lot number: 0526023-00)
e) Reason for selection
EPI-200 SCT kit is recommended in the test method.
f) Storage conditions
The tissue insert and the medium were stored in a cold place in the cell experimental room No. 1 (permissible range: from 1°C to 10°C). Nylon mesh and Phosphate buffered saline without Ca^{2+} and Mg^{2+} were stored at room temperature in the cell experimental room No. 1.
g) Quality of reconstructed human epidermis (RHE) model
The results of quality verification (biological contaminants, tissue viability, barrier function and sterility) performed by the manufacturer are shown in Appendix 1.

12.3 Culture Condition (Setting value)

Incubator	CO_2 incubator (MCO-18AIC, SANYO Electric)
Temperature	37°C
Humidity	Under humid condition
CO_2 concentration	5%

12.4 Buffer Solution, Medium Containing MTT Solution and MTT Extraction Solvent

a) Buffer solution

Phosphate buffered saline without Ca^{2+} and Mg^{2+} (pH 7.0) (PBS(−))

b) Medium containing MTT solution

1) Preparation method

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Lot number: LC082, for research, DOJINDO Laboratories) was dissolved in PBS(−) to prepare 5 mg/mL MTT solution. This solution was diluted with the medium to prepare medium containing 1 mg/mL MTT solution (MTT medium).

2) Timing of preparation and storage conditions

MTT medium was prepared just before use. MTT medium was stored at room temperature under light shielding until use.

c) MTT extraction solvent

2-Propanol (Lot number: TWG2194, Special grade, Wako Pure Chemical Industries)

13. TEST PROCEDURE

13.1 Preliminary Test

a) Test for reactivity with MTT

Twenty five milligrams of the test substance and 1 mL of MTT medium were mixed, the mixture was incubated for 60 minutes. After the incubation, the change in color of the MTT medium was evaluated. As a result, the change in color was not observed and it was judged that the test substance had no reactivity with MTT. Therefore, interference of the test substance with MTT (interference test) was not evaluated in the skin corrosion test.

13.2 Skin Corrosion Test

Duplicate tissue inserts were used for the test substance, negative control substance and positive control substance, respectively. Duplicate tissue inserts were used to check the tissue-binding of the test substance (tissue-binding test).

a) Pre-incubation

1) Tissue inserts were placed in a 6-well plate (Asahi Glass) filled with 0.9 mL/well of the medium and incubated for 60 ± 5 minutes.

b) Exposure of the test substance

1) At 60-minute exposure, medium was removed from all wells and 0.9 mL/well of fresh medium was added.

2) 3-minute and 60-minute exposures were conducted. Twenty five milligrams of the

test substance and 50 μL of the control substances were applied onto each tissue surface at 45 second interval. For the test substance, 25 μL of distilled water was added to each tissue surface just before the exposure. For the control substances, a nylon mesh was placed on each tissue surface to spread the control substances over the tissue surface. The 45 second interval allowed sufficient time for both application and washing procedures at the end of the exposure period.

3) At 3-minute exposure, each plate was placed at room temperature until 3 minutes was completed for the first exposed tissue insert in each plate. At 60-minute exposure, each plate was placed into the incubator until 60 ± 1 minutes was completed for the first exposed tissue insert in each plate.

c) Rinsing

1) After the exposure, each tissue insert was rinsed approximately twenty times with PBS(−).
2) Inside and outside of the tissue inserts were wiped. The tissue inserts were placed into new 24-well plates (Corning) filled with 300 μL/well of fresh medium. Remaining PBS(−) was completely removed from the tissue surface.

d) MTT reaction and extraction

1) All tissue inserts were transferred into a 24-well plate filled with 0.3 mL/well of MTT medium and incubated for 180 ± 5 minutes.
2) MTT medium was removed from all wells. The outside of tissue inserts were washed three times with PBS(−).
3) All tissue inserts were transferred into a new 24-well plate. Two milliliters per well of 2-propanol was added to the tissue insert.
4) The plate was put into a plastic bag, and extraction was performed at room temperature for 2 hours or more using a plate shaker.
5) The extracts in the tissue inserts were transferred into the wells and homogenized.

e) Measuring of optical density (OD) and calculation of cell viability

1) Two hundred microliters per well of the extracts were transferred into a 96-well plate (Corning) (n = 3). Two hundred microliters per well of 2-propanol was used as blank (n = 6).
2) OD of each extract was measured spectrophotometrically using Multimode Microplate Reader (FLUOstar OPTIMA, BMG LABTECH) at 570 nm.
3) The mean of blank OD was subtracted from ODs of each tissue insert and the mean value was calculated in each tissue insert to obtain OD of each tissue insert. The cell viability of each tissue insert was calculated by the following formula.

$$\text{Cell viability (\%)} = \frac{\text{OD of each tissue insert of each treatment group}}{\text{Mean OD of the negative control substance group}} \times 100$$

The mean cell viability of each treatment group was calculated from the cell viability of each tissue insert.

f) Tissue-binding test

The tissue-binding test was carried out using the same procedure as described in 13.2 a) to e), except medium without MTT was used instead of MTT medium. After the measuring of OD, the staining ratio was calculated by the following formula.

$$\text{Staining ratio (\%)} = \frac{\text{Mean OD of the test substance group (without MTT)}}{\text{Mean OD of the negative control substance group (with MTT)}} \times 100$$

Since the staining ratios were below 5%, the ODs were not corrected.

14. JUDGEMENT CRITERIA OF THE RESULTS

Skin corrosion was judged according to the following criteria.

Mean cell viability	Category
< 25% (3-minute exposure)	Corrosive (UN GHS*1 Category 1A)
≥ 25%, < 50% (3-minute exposure)	Corrosive (UN GHS*1 Category 1B and 1C)
≥ 50% (3-minute exposure) and < 15% (60-minute exposure)	
≥ 50% (3-minute exposure) and ≥ 15% (60-minute exposure)	Non-corrosive (UN GHS*1 Category 2 or not classified)

*1: Globally Harmonized System of Classification and Labelling of Chemicals

15. ACCEPTABLE CRITERIA OF THE TEST

a) Mean OD in the negative control substance group is ≥ 0.8 and ≤ 2.8.

b) Mean cell viability in the positive control substance group in the 60-minute exposure is < 15%.

c) For the substance which the cell viability is 20 to 100%, coefficient of variation (CV) between the two tissue inserts is 30% or below.

16. DEVIATION FROM THE STUDY PLAN

There were no deviations from the study plan.

17. TEST RESULTS AND DISCUSSION

The test results are shown in Tables 1 and 2.

As a result of tissue-binding test in the 3-minute and 60-minute exposures, the staining ratios

of the tissue insert treated by the test substance were 0.5% and 0.3%, respectively, and they were below 5%. Therefore, the ODs were not corrected.

ODs in the negative control substance group in the 3-minute and 60-minute exposures were 1.941 and 2.047, respectively. The mean cell viability in the positive control substance group in the 60-minute exposure was 1.4%. Coefficient of variation (CV) which the cell viability was from 20% to 100% were 30% or below for all substances. These results indicated that the present study was appropriately performed.

As a result of the skin corrosion test, the cell viabilities treated by APFHx in the 3-minute and 60-minute exposures were 94.4% and 5.0%, respectively.

18. CONCLUSION

It is concluded that APFHx was "Corrosive" (UN GHS Category 1B and 1C) under the present test conditions.

Table 1 Results of skin corrosion test

Group	Tissue No.	3-minute exposure OD[a]			Cell viability (%)[b]		SD[c]	CV	60-minute exposure OD[a]			Cell viability (%)[b]		SD[c]	CV	Category
			Mean			Mean				Mean			Mean			
Negative control (Distilled water)	1	1.937							2.026							
		1.937	1.939		99.9				2.031	2.030		99.2				
		1.942		1.941		100	0.14	0.1	2.032		2.047		100	1.13	1.1	
	2	1.938							2.070							
		1.945	1.942		100.1				2.063	2.063		100.8				
		1.942							2.056							
Positive control (8N Potassium hydroxide solution)	1	0.113							0.029							
		0.111	0.112		5.8				0.029	0.029		1.4				
		0.113		0.111		5.7	0.14	2.5	0.030		0.028		1.4	0.07	5.1	
	2	0.108							0.025							
		0.111	0.109		5.6				0.025	0.026		1.3				
		0.109							0.027							
Test substance (APFHx)	1	1.680							0.103							
		1.669	1.671		86.1				0.104	0.103		5.0				
		1.665		1.833		94.4	11.74	12.4	0.103		0.102		5.0	0.07	1.4	Corrosive
	2	1.983							0.095							
		1.997	1.994		102.7				0.109	0.100		4.9				
		2.002							0.096							

OD: optical density, SD: standard deviation, CV: coefficient of variation

a) Value of OD which the mean of blank OD was subtracted from was shown.

b) Cell viability in the negative control substance was regarded as 100%.

c) The SD was calculated from the cell viabilities of each tissue insert (n=2).

Table 2 Results of tissue-binding test

Group	Tissue No.	3-minute exposure OD[a)]	3-minute exposure OD[a)] Mean	3-minute exposure Staining ratio[b)] (%)	60-minute exposure OD[a)]	60-minute exposure OD[a)] Mean	60-minute exposure Staining ratio[b)] (%)
Test substance[c)] (APFHx)	1	0.007	0.012	0.5	0.005	0.005	0.3
		0.021			0.004		
		0.009			0.006		
		0.005	0.009		0.005	0.006	
	2	0.005	0.006		0.008	0.007	
		0.007			0.007		

OD: optical density

a) Value of OD which the mean of blank OD was subtracted from was shown.

b) Staining ratio (%) = $\frac{\text{Mean OD of the test substance group (without MTT)}}{\text{Mean OD of the negative control substance group (with MTT)}} \times 100$

c) Medium without MTT was used instead of MTT medium.

Appendix 1 Certificate of analysis

Certificate of Analysis

MatTek CORPORATION

Product: EpiDerm™ Reconstructed Human Epidermis

Lot Number: 28302

Part#: EPI-200, EPI-212

Description: Reconstructed human epidermis tissue containing normal human keratinocytes. This product is for research use only. Not for use in animals, humans or diagnostic purposes.

I. Cell source

All cells used to produce EpiDerm™ are purchased or derived from tissue obtained by MatTek Corporation from accredited institutions. In all cases, consent was obtained by these institutions from the donor or the donor's legal next of kin, for use of the tissues or derivatives of the tissue for research purposes.

Keratinocyte Strain: 4F0219

II. Analysis for potential biological contaminants

The cells used to produce EpiDerm™ tissue are screened for potential biological contaminants. Tests for each potential biological contaminant listed below were performed according to the test method given. Results of "Not detected" indicate that testing for the potential biological contaminant was not observed as determined by the stated test method.

Keratinocytes:	
HIV-1 virus - Oligonucleotide-directed amplification	Not detected
Hepatitis B virus - Oligonucleotide- directed amplification	Not detected
Hepatitis C virus - Oligonucleotide- directed amplification	Not detected
Bacteria, yeast, and other fungi - long term antibiotic, antimycotic free culture	Not detected

III. Analysis for tissue functionality and quality

Test	Specification	Acceptance criteria	Result and QA Statement	
Tissue viability	MTT QC assay, 4 hours, n=3	OD (540-570 nm) [1.0-3.0]	1.708 ± 0.063	Pass
Barrier function	ET-50 assay, 100 µL 1% Triton X-100, 4 time-points, n=3, MTT assay	ET-50 [3.68-8.02 hrs]	6.42 hrs	Pass
Sterility	Long term antibiotic and antimycotic free culture	No contamination	Sterile	Pass

Tissue viability and the barrier function test are within the acceptable ranges and indicate appropriate formation of the epidermal barrier, the presence of a functional stratum corneum, a viable basal cell layer, and intermediate spinous and granular layers. Results obtained with this lot conform to the requirements of the **OECD TG 431 and TG 439.**

Initials: PN

Date: 3/28/18

March 28, 2018

Date

Quality Assurance Associate

CAUTION: Whereas all information herein is believed to be correct, no absolute guarantee that human derived material is non-infectious can be made or is implied by this certificate of analysis. All tissues should be treated as potential pathogens. The use of protective clothing and eyeware and appropriate disposal procedures are strongly recommended.

MatTek Corporation
200 Homer Avenue, Ashland, MA - USA
+1-508-881-6771

www.mattek.com
information@mattek.com

Appendix 2 Historical data in the testing facility

	OD			
	3-minute exposure		60-minute exposure	
	Negative control	Positive control	Negative control	Positive control
Mean	1.900	0.206	1.852	0.065
SD	0.192	0.069	0.191	0.031
Max	2.331	0.371	2.176	0.113
Min	1.635	0.028	1.573	0.024
Number of test	20		20	
Test period	January, 2016 - February, 2018			

OD: optical density

SD: standard deviation

Appendix 3 Results of demonstration of proficiency in the testing facility

Chemical Name	CAS number	Manufacture name	Lot number	GHS category	Results in the testing facility			
					Cell viability(%)		GHS category	
					3-minute exposure	60-minute exposure		
Bromoacetic acid	79-08-3	Wako Pure Chemical Industries	PDP1253	1A	4.3		1A	Pass
Boron trifluoride dihydrate	13319-75-0	SIGMA-ALDRICH	BCBM0579V	1A	8.4		1A	Pass
Phenol	108-95-2	Wako Pure Chemical Industries	AWE3062	1A	23.8	11.0	1A	Pass
Dichloroacetyl chloride	79-36-7	Wako Pure Chemical Industries	LAF3054	1A	0.6	0.8	1A	Pass
Glyoxylic acid monohydrate	563-96-2	Wako Pure Chemical Industries	PDF4037	1B-and-1C	62.1	1.3	1B-and-1C	Pass
Lactic acid	598-82-3	Wako Pure Chemical Industries	KPQ4270	1B-and-1C	72.2	3.4	1B-and-1C	Pass
Ethanolamine	141-43-5	Wako Pure Chemical Industries	KPH4499	1B	67.1	8.7	1B-and-1C	Pass
Hydrochloric acid (14.4%)	7647-01-0	Wako Pure Chemical Industries	CTR5848	1B-and-1C	71.2	6.5	1B-and-1C	Pass
Phenethyl bromide	103-63-9	Wako Pure Chemical Industries	WEF3308	NC		97.5	NC	Pass
4-Amino-1,2,4-triazole	584-13-4	Wako Pure Chemical Industries	AWJ0904	NC		80.6	NC	Pass
4-(methylthio)-benzaldehyde	3446-89-7	Wako Pure Chemical Industries	TLK3470	NC		100.4	NC	Pass
Lauric acid	143-07-7	Wako Pure Chemical Industries	CTQ1343	NC		74.7	NC	Pass

QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: *In vitro* Skin Corrosion Test of APFHx Using EpiDerm™ SCT (EPI-200)

Study Number: K10-0379

I assure that the final report accurately describes the test methods and procedures, and that the reported results accurately reflect the raw data of this study. The inspections of the 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 23, 2018	March 23, 2018
Cell pre-culture	March 28, 2018	March 28, 2018
Exposure of test substance	March 28, 2018	March 28, 2018
MTT assay	March 28, 2018	March 28, 2018
Raw data and draft final report	April 25, 2018	April 25, 2018
Final Report	May 11, 2018	May 11, 2018

Date: May 11, 2018

Quality Assurance Manager: ___