CERI

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Study Number	K01-6462

FINAL REPORT

REVERSE MUTAGENICITY TEST OF
APFHx(C-1500N)
ON BACTERIA

April, 2017

Chemicals Evaluation and Research Institute, Japan, Hita

GLP STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: Reverse Mutagenicity Test of APFHx(C-1500N) on Bacteria

Study Number: K01-6462

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: _____ April 3, 2017
Date

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QUALITY ASSURANCE STATEMENT

Corrected page

1. TITLE

Reverse Mutagenicity Test of APFHx(C-1500N) on Bacteria

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 mutations was investigated by using *Salmonella typhimurium* and *Escherichia coli*.

5. TEST METHOD

"OECD Guidelines for the Testing of Chemicals, No. 471, Bacterial Reverse Mutation Test" (Adopted: July 21, 1997)

6. GLP PRINCIPLE

OECD Principles of Good Laboratory Practice, November 26, 1997

7. DATES

Study Initiation Date	February 28, 2017
Experiment Starting Date	March 2, 2017
Experiment Completion Date	March 13, 2017
Study Completion Date	April 3, 2017

8. PERSONNEL CONCERNED WITH STUDY

Study Director:

Section 3, CERI Hita

Study Staff:

Section 3, CERI Hita
(Preparation of the test substance solution, treatment, observation and counting of the revertant colony)

9. STORAGE OF TEST ITEMS, RAW DATA, ETC.

The original study plan, the original final report, the raw data, documents concerning the study presented by the sponsor, other reports are stored in the archives of the testing facility.
The storage period is 10 years after the study completion date.
The management of these items after the storage period (continuation of storage, disposal or return) will be discussed with the sponsor.

10. APPROVAL BY AUTHOR

Study Director: April 3, 2017
Date

11. SUMMARY

The ability of APFHx(C-1500N) to induce mutations was investigated using *Salmonella typhimurium* strains TA100, TA1535, TA98 and TA1537 and *Escherichia coli* strain WP2*uvrA* with a pre-incubation method in the presence and absence of a metabolic activation system (S9 mix).

The mutagenicity of the test substance was judged to be negative because the numbers of revertant colonies in the test substance treatment groups for all tester strains were less than twice that in each negative control.

Consequently, it was concluded that APFHx(C-1500N) had no ability to induce mutations under the present test conditions.

12. MATERIALS

12.1 Test Substance and Control Substances

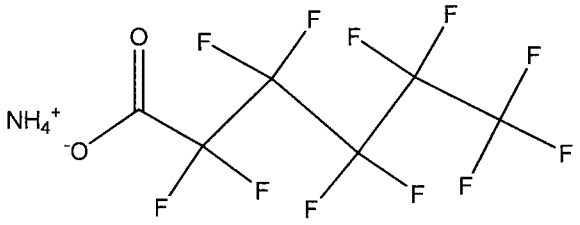
a) Test substance (information provided by the sponsor)

1) Chemical name, etc.

Chemical name	2,2,3,3,4,4,5,5,6,6,6-undecafluorohexanoic acid, ammonium salt
Other name	APFHx(C-1500N)
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	50%
Impurity	Water: 50%
Supplier	DAIKIN INDUSTRIES, LTD.
Lot number	C150E62004

4) Physicochemical properties

Appearance at ordinary temperature Colorless transparent liquid

Stability Stable in storage condition

Solubility to vehicle, etc.

Vehicle	Solubility	Stability in vehicle
Water	≥50.0 mg/mL*1	Stable*2
Dimethyl sulfoxide (DMSO)	—	—
Acetone	—	—

*1: Confirmed at the testing facility.

*2: The test substance solution of 50.0 mg/mL prepared with distilled water was considered to be stable from the facts that there were no change in color, exothermic reaction nor gas generation at room temperature within 4 hours after preparation (confirmed at the testing facility).

5) Storage conditions

The test substance was put into light-resistant airtight container and stored in the test substance storage room at room temperature (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 (vehicle)

1) Name

Distilled water

2) Manufacturer, lot number and grade

Manufacturer	Otsuka Pharmaceutical Factory
Lot number	K6C70
Grade	For injection

3) Reason for selection of vehicle

The test substance was soluble in distilled water at 50.0 mg/mL. The test substance solution of 50.0 mg/mL prepared with distilled water was considered to be stable from the facts that there were no change in color, exothermic reaction nor gas generation at room temperature within 4 hours after preparation. Therefore, distilled water was select as a vehicle.

4) Storage conditions

Negative control substance was stored in a room temperature in the preparation room No. 2.

c) Positive control substances

1) Name, etc.

Name	Manufacturer	Lot No.	Appearance	Purity	Grade
AF-2†1	Wako Pure Chemical Industries	STQ3987	Yellowish red crystalline powder	99.7%	Special grade
NaN_3†2	Wako Pure Chemical Industries	MPG7000	White crystalline powder	100.4%	Special grade
ICR-191	Polysciences	562079	Yellow-orange crystalline powder	–	–
2AA†3	Wako Pure Chemical Industries	CTK0326	Yellow-green brown powder	96.7%	–

†1: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

†2: Sodium azide

†3: 2-Aminoanthracene

2) Storage conditions

Positive control substances were put into light-resistant airtight containers and stored in a cold place in the test substance storage room (permissible range: from 1 to 10°C).

3) 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.

12.2 Bacterial Strains

a) Strains and reason for selection

Salmonella typhimurium (*S. typhimurium*) strains TA100, TA1535, TA98 and TA1537 and *Escherichia coli* (*E. coli*) strain WP2*uvrA* were used. *S. typhimurium* strains and *E. coli* strain were supplied from National Institute of Health Sciences on August 6, 2014. These tester strains have been recommended to use for the Reverse Mutagenicity Test on Bacteria in the "OECD Guidelines for the Testing of Chemicals, No. 471, Bacterial Reverse Mutation Test ".

b) Storage

Amino acid requirement, sensitivity to ultraviolet-rays, *rfa* membrane mutation, presence or absence of plasmid pKM101 and each number of revertant colonies of negative and positive controls were preliminarily assessed in the testing facility. DMSO was added to fresh overnight cultures of the tester strains that had been examined these characteristics at a volume ratio of 0.9 to 10. The mixture was frozen as a stock culture in the Ames test room No. 1 below −80°C until use. The mixture was thawed just before use.

12.3 Medium and S9 Mix

a) Medium

1) Minimal glucose agar plate

Tesmedia AN (Lot No. ANI630KF, manufactured on November 24, 2016, Oriental Yeast) was used.

2) Soft agar

A solution containing 0.5 mM histidine and 0.5 mM biotin for *S. typhimurium* strains or 0.5 mM tryptophan for *E. coli* strain was added to a soft agar solution containing 0.6 w/v% agar (Bacto Agar, Lot No. 4045079, Becton, Dickinson and Company) and 0.5 w/v% NaCl at a volume ratio of 1 to 10.

b) S9 mix

1) Rat liver S9

S9 (Lot No. 16111809, manufactured on November 18, 2016, Oriental Yeast) prepared from the liver of 7-week-old male SD rats (body weight: 205.1±8.9 g) administered with phenobarbital (one time at 30 mg/kg and three times at 60 mg/kg) and 5,6-benzoflavone (one time at 80 mg/kg) was used. The S9 was cryopreserved in the Ames test room No. 1 below −80°C until use. S9 was thawed just before use.

2) Composition of S9 mix

S9 mix was prepared just before use. One milliliter of S9 mix consisted of 8 μmol $MgCl_2$, 33 μmol KCl, 5 μmol Glucose-6-phosphate, 4 μmol NADPH, 4 μmol NADH, 100 μmol sodium-phosphate buffer (pH 7.4) and 0.1 mL of S9.

12.4 Pre-cultures of the Tester Strains

Frozen stock culture of bacterial strains, 20 μL for TA100, TA1535 and TA98, 50 μL for TA1537, 5 μL for WP2*uvrA*, were respectively inoculated to 11 mL of Nutrient broth No. 2 (Lot No. 1347750, OXOID) in L-tube (volume: 27 mL). The culture was incubated at 37±0.5°C for 10 hours with shaking at about 50 times/minute by a seesaw type of shaker (MONOSIN-IIA, TAITEC).

The number of viable cells was calculated from O.D. value at 660 nm measured by a photometer (miniphoto 518R, TAITEC) at the end of incubation. It was confirmed that the numbers of viable cells were more than 1.0×10^9 cells/mL.

The final numbers of viable cells are shown below:

Test	TA100	TA1535	WP2*uvrA*	TA98	TA1537
Main test-1	2.9	3.2	6.9	4.5	3.7
Main test-2	2.5	3.2	6.6	4.7	3.4

($\times10^9$ cells/mL)

12.5 Preparation of Test Substance Solution and Positive Control Substance Solutions

a) Preparation of test substance solution

1) Preparation method

The test substance was weighed, corrected by the purity, added distilled water and mixed with a laboratory mixer to make a 50.0 mg/mL of original solution. The test substance solutions of each required concentration were prepared with the same vehicle.

2) Timing of preparation

The test substance solutions were prepared just before use, kept under yellow light at room temperature and used within 4 hours after preparation.

b) Preparation of positive control substance solutions

1) Preparation method and storage conditions

NaN_3 was dissolved in distilled water. AF-2, ICR-191 and 2AA were dissolved in DMSO. These positive control substance solutions were stored in the Ames test room No. 1 below −80°C.

2) Timing of preparation

The positive control substance solutions were thawed just before use.

13. METHODS

This study was performed by the pre-incubation method with and without S9 mix. Triplicate plates were used for the negative control, the positive control and the test substance treatment groups. The study number, name of the tester strain, the presence or absence of S9 mix and the dose level were noted on each plate.

13.1 Procedures

After 0.1 mL of the test substance solution and vehicle or positive control substance solutions, 0.5 mL of 0.1 M sodium phosphate buffer (pH 7.4) or S9 mix, and 0.1 mL of the bacterial culture were added to a test tube, the mixture was shaken at 37±0.5°C for 20 minutes. Two milliliters of the soft agar were then added to each tube and the mixture was poured onto a minimal glucose agar plate. The number of revertant colonies was counted after incubation at 37±0.5°C for 48 hours.

13.2 Confirmation of Sterility

The highest concentration of the test substance solution (0.1 mL) and S9 mix (0.5 mL) were individually mixed with 2 mL of the soft agar and were poured onto each minimal glucose agar plate in order to examine bacterial contamination. Bacterial contamination was judged with those plates after incubation at 37±0.5°C for 48 hours.

13.3 Negative Control and Positive Controls

The vehicle was employed as a negative control. The positive controls and their doses for each tester strains are shown below:

	TA100	TA1535	WP2*uvrA*	TA98	TA1537
−S9 mix	AF-2	NaN_3	AF-2	AF-2	ICR-191
	0.01	0.5	0.01	0.1	0.5
+S9 mix	2AA	2AA	2AA	2AA	2AA
	1	2	10	0.5	2

(Unit: μg/plate)

13.4 Dose Selection

a) Main test-1

A total of six doses including 5000 μg/plate as the highest dose and five lower doses diluted with a geometric progression of 4 were set.

b) Main test-2

The results of main test-1 showed that the number of revertant colonies in the test substance treatment groups was less than twice that in each negative control for all tester strains with and without S9 mix. The bacterial growth inhibition was not observed at any test condition. The precipitation of the test substance was not observed in either the presence or absence of S9 mix.

Therefore, the highest dose was selected at 5000 μg/plate and four lower doses of 2500, 1250, 625 and 313 μg/plate diluted with a geometric progression of 2 were employed for all tester strains with and without S9 mix in the main test-2.

13.5 Observation and Colony Counting

a) Observation

The precipitation of the test substance was observed macroscopically and the bacterial growth inhibition was observed by using a stereomicroscope at the end of the incubation.

b) Colony counting

All plates were counted with a colony analyzer (CA-11D, SYSTEM SCIENCE). Square correction and miscounting correction were conducted when counting with the colony analyzer.

14. JUDGEMENT CRITERIA OF TEST RESULTS

The test substance was judged to be positive when the number of revertant colonies increased to twice or more than that in the negative control and when the responses were dose-related and/or reproducible. The other cases were judged to be negative. No statistical methods were used.

15. DEVIATION FROM THE PROTOCOL

There were no deviations from the protocol.

16. TEST RESULTS

16.1 Main Test-1

The test results are shown in Table 1 and the dose-response curves are shown in Figs. 1 and 2.

The number of revertant colonies in the test substance treatment groups was less than twice that in each negative control for all tester strains with and without S9 mix.

The bacterial growth inhibition was not observed at any test condition. The precipitation of the test substance was not observed in either the presence or absence of S9 mix.

16.2 Main Test-2

The test results are shown in Table 2 and the dose-response curves are shown in Figs. 3 and 4.

The number of revertant colonies in the test substance treatment groups was less than twice that in each negative control for all tester strains with and without S9 mix.

The bacterial growth inhibition was not observed at any test condition. The precipitation of the test substance was not observed in either the presence or absence of S9 mix.

17. DISCUSSION

The mutagenicity of the test substance was judged to be negative because the number of revertant colonies in the test substance treatment groups was less than twice that in the negative control for all tester strains regardless of the presence or absence of S9 mix.

The numbers of revertant colonies in the positive controls were above twice that in the negative controls. The test results showed that the numbers of revertant colonies in the negative and positive controls were within the range of the historical data at the testing facility. It was also confirmed that the test system was free from bacterial contamination, which declares the test results to be valid.

18. CONCLUSION

It was concluded that APFHx(C-1500N) had no ability to induce mutations under the present test conditions.

Table 1 Results of the main test-1

Test substance: APFHx(C-1500N)

Test Period		From March 2, 2017 to March 6, 2017				
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	121 139 (133 ± 10) 139	10 15 (13 ± 3) 13	26 40 (32 ± 7) 29	14 18 (16 ± 2) 15	14 20 (16 ± 3) 15
	4.88	155 121 (136 ± 17) 133	12 4 (9 ± 5) 12	39 38 (34 ± 8) 25	15 18 (15 ± 4) 11	15 17 (16 ± 1) 17
	19.5	117 126 (119 ± 7) 113	14 10 (14 ± 4) 18	42 41 (37 ± 8) 27	17 13 (15 ± 2) 15	26 11 (18 ± 8) 17
	78.1	119 124 (130 ± 15) 147	7 11 (9 ± 2) 8	41 38 (39 ± 2) 38	18 13 (17 ± 3) 19	27 17 (19 ± 7) 14
	313	133 150 (136 ± 13) 124	5 10 (10 ± 5) 15	43 34 (36 ± 6) 32	13 18 (17 ± 3) 19	26 7 (16 ± 10) 14
	1250	150 145 (149 ± 4) 152	8 8 (10 ± 4) 15	32 22 (31 ± 9) 39	24 22 (21 ± 4) 17	14 14 (16 ± 3) 20
	5000	123 155 (132 ± 20) 119	10 22 (16 ± 6) 15	38 26 (34 ± 7) 38	14 26 (18 ± 7) 13	13 15 (15 ± 3) 18
+S9 mix	Negative control	133 126 (139 ± 17) 159	8 10 (10 ± 2) 12	27 29 (29 ± 3) 32	21 26 (24 ± 3) 24	17 12 (20 ± 10) 31
	4.88	113 115 (119 ± 9) 129	19 18 (15 ± 6) 8	26 33 (33 ± 7) 39	24 29 (24 ± 5) 20	18 17 (16 ± 3) 12
	19.5	133 146 (134 ± 12) 122	17 11 (14 ± 3) 14	46 33 (35 ± 11) 25	25 19 (24 ± 5) 29	22 17 (20 ± 3) 20
	78.1	124 127 (135 ± 16) 153	8 6 (6 ± 2) 5	29 35 (37 ± 10) 48	45 15 (31 ± 15) 33	21 15 (18 ± 3) 19
	313	127 133 (126 ± 8) 117	13 13 (12 ± 1) 11	31 39 (34 ± 4) 33	22 32 (27 ± 5) 28	11 17 (17 ± 7) 24
	1250	128 113 (117 ± 10) 110	7 7 (7 ± 1) 8	39 29 (32 ± 6) 27	19 20 (20 ± 1) 20	20 14 (22 ± 10) 33
	5000	119 127 (113 ± 17) 94	8 11 (8 ± 4) 4	38 42 (40 ± 2) 39	20 29 (26 ± 5) 29	18 20 (22 ± 5) 27
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(μg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	928 949 (899 ± 70) 819	326 383 (361 ± 31) 375	157 173 (164 ± 8) 163	499 487 (478 ± 27) 447	701 692 (720 ± 42) 768
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	1524 1567 (1517 ± 53) 1461	381 362 (368 ± 11) 361	620 686 (627 ± 56) 575	535 484 (506 ± 26) 500	252 284 (250 ± 35) 215

[Note]

( ): The mean number of colonies per plate ± standard deviation (n=3).

AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

NaN_3: Sodium azide

2AA: 2-Aminoanthracene

Table 2 Results of the main test-2

Test substance: APFHx(C-1500N)

Test Period		From March 9, 2017 to March 13, 2017				
With(+)or without(-) S9 mix	Test substance dose (μg/plate)	Number of revertant colonies per plate				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Negative control	117 138 (131 ± 12) 139	13 18 (13 ± 5) 8	35 42 (38 ± 4) 38	18 12 (18 ± 6) 24	24 22 (22 ± 2) 21
	313	119 151 (123 ± 26) 100	13 11 (12 ± 1) 11	24 26 (30 ± 8) 39	34 26 (26 ± 8) 19	21 19 (19 ± 2) 17
	625	119 152 (144 ± 23) 162	17 11 (14 ± 3) 13	41 36 (34 ± 8) 25	18 22 (21 ± 3) 24	10 25 (19 ± 8) 21
	1250	135 126 (142 ± 20) 164	6 10 (9 ± 2) 10	40 22 (34 ± 10) 39	17 17 (19 ± 4) 24	19 22 (22 ± 3) 24
	2500	144 144 (143 ± 2) 140	5 7 (9 ± 5) 15	39 25 (29 ± 8) 24	24 20 (20 ± 5) 15	21 17 (18 ± 2) 17
	5000	127 156 (133 ± 20) 117	10 12 (10 ± 2) 8	27 24 (30 ± 8) 39	19 11 (17 ± 5) 21	12 21 (17 ± 5) 17
+S9 mix	Negative control	107 122 (114 ± 8) 112	14 8 (11 ± 3) 11	28 38 (36 ± 7) 41	41 20 (29 ± 11) 27	25 25 (25 ± 0) 25
	313	123 100 (109 ± 13) 103	5 13 (8 ± 5) 5	22 36 (31 ± 8) 34	25 32 (28 ± 4) 27	33 20 (27 ± 7) 27
	625	103 138 (123 ± 18) 128	7 7 (9 ± 3) 12	21 39 (33 ± 10) 38	22 26 (30 ± 10) 41	28 33 (27 ± 6) 21
	1250	128 124 (128 ± 4) 132	8 7 (11 ± 6) 17	32 48 (39 ± 8) 38	14 22 (21 ± 7) 28	24 35 (27 ± 7) 22
	2500	118 109 (114 ± 5) 115	10 7 (8 ± 2) 6	35 41 (37 ± 3) 35	28 33 (29 ± 4) 26	28 17 (21 ± 6) 17
	5000	90 119 (114 ± 22) 134	14 10 (9 ± 6) 3	38 41 (35 ± 8) 26	20 28 (26 ± 5) 29	19 18 (26 ± 14) 42
Positive control -S9 mix	Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Dose(μg/plate)	0.01	0.5	0.01	0.1	0.5
	Number of revertant colonies/plate	1012 922 (969 ± 45) 974	289 329 (324 ± 32) 353	162 153 (157 ± 5) 156	615 573 (575 ± 39) 538	599 668 (658 ± 55) 708
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	1705 1703 (1757 ± 91) 1862	387 349 (385 ± 35) 419	550 574 (585 ± 42) 632	453 507 (485 ± 29) 496	312 245 (274 ± 35) 264

[Note]

( ): The mean number of colonies per plate ± standard deviation (n=3).

AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

NaN_3: Sodium azide

2AA: 2-Aminoanthracene

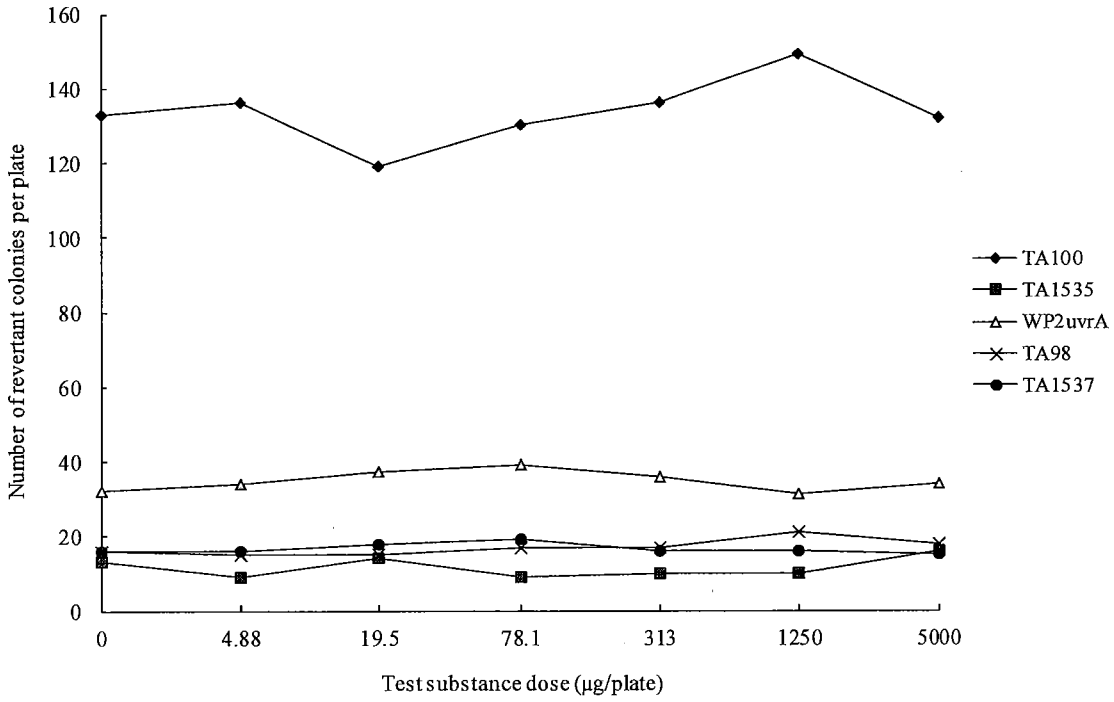


Fig. 1 Dose response curve of APFHx(C-1500N) without S9 mix in the main test-1

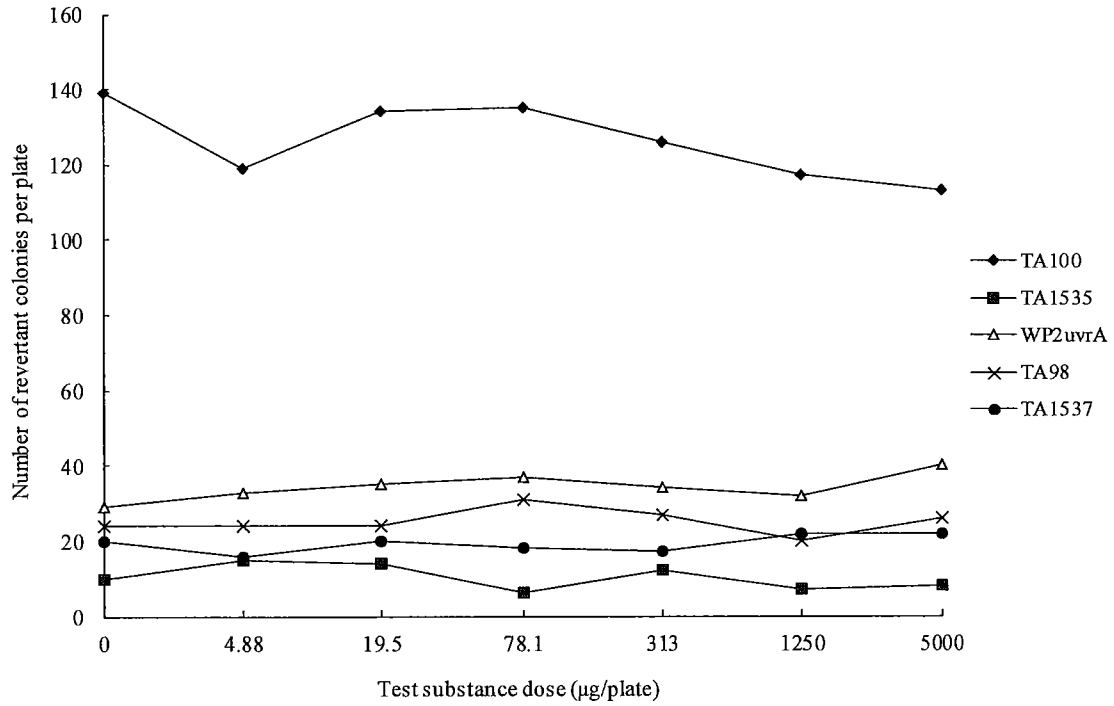


Fig. 2 Dose response curve of APFHx(C-1500N) with S9 mix in the main test-1

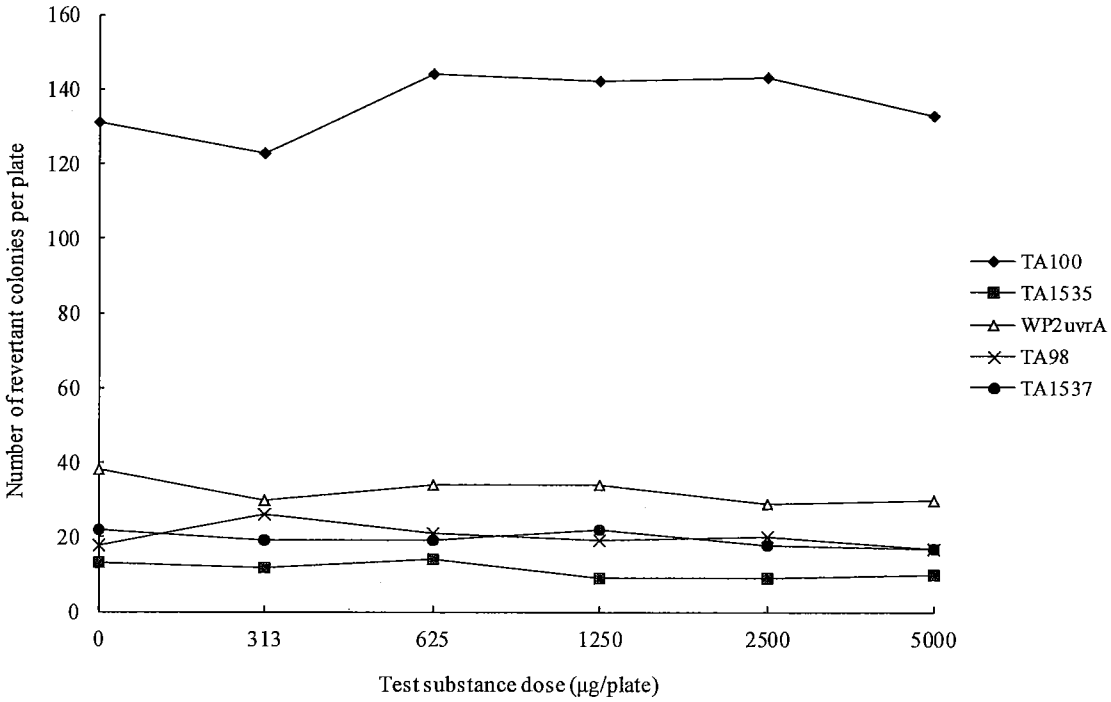


Fig. 3 Dose response curve of APFHx(C-1500N) without S9 mix in the main test-2

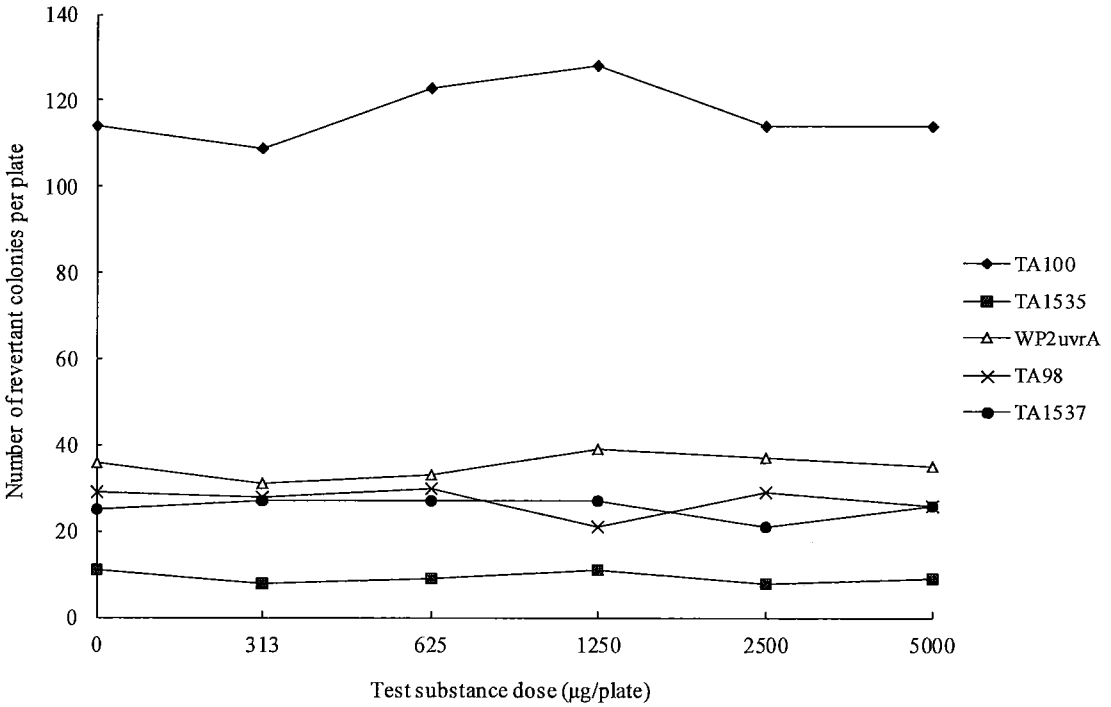


Fig. 4 Dose response curve of APFHx(C-1500N) with S9 mix in the main test-2

Appendix 1 Historical data

Negative Control (Mean±3S.D.)

	-S9 mix					+S9 mix				
	TA100	TA1535	WP2*uvrA*	TA98	TA1537	TA100	TA1535	WP2*uvrA*	TA98	TA1537
Mean	119	11	32	20	12	124	11	31	29	14
S.D.	25	4	10	6	6	23	4	10	8	6
Upper Limit	194	23	62	38	30	193	23	61	53	32
Lower Limit	44	1	2	2	1	55	1	1	5	1

Positive Control (Mean±3S.D.)

	-S9 mix					+S9 mix				
	TA100	TA1535	WP2*uvrA*	TA98	TA1537	TA100	TA1535	WP2*uvrA*	TA98	TA1537
Chemical	AF-2	NaN_3	AF-2	AF-2	ICR-191	2AA	2AA	2AA	2AA	2AA
Dose (μg/plate)	0.01	0.5	0.01	0.1	0.5	1	2	10	0.5	2
Mean	854	312	112	498	635	1561	292	379	397	207
S.D.	104	57	21	54	103	194	35	101	40	39
Upper Limit	1166	483	175	660	944	2143	397	682	517	324
Lower Limit	542	141	49	336	326	979	187	76	277	90

[Notes]

- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- 2AA: 2-Aminoanthracene
- Test period: From September, 2016 to February, 2017
- Tesmedia AN

Appendix 2 Assay data of S9

ORIENTAL YEAST CO., LTD.

50 KANO-CHO, NAGAHAMA-SHI, SHIGA 526-0804 JAPAN
PHONE: 0749-64-2346 FAX: 0749-63-7910

ASSAY DATA

S 9

Rat liver 9,000 × *g* supernatant fraction induced with phenobarbital and 5,6-benzoflavone

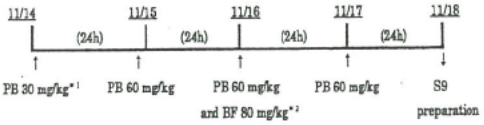
Lot No. : 16111809

Date prepared : November 18, 2016

Animals :

Strain	Sprague-Dawley rat (Crl:CD(SD))
Age, Sex, Numbers	7 weeks, Male, 45rats
Body weight	205.1 ± 8.9 g(Mean ± S.D.)

Induction (phenobarbital and 5,6-benzoflavone, i.p.) :



＊1 : PB = phenobarbital ＊2 : BF = 5,6-benzoflavone

Bacterial contamination (500 μL S9/Minimal agar plate) : NEGATIVE

Biochemical properties :

S9 fraction

Protein content	20.5 mg/mL S9 (53.5 mg/g liver)

Microsome fraction*1

Protein content	14.6 mg/mL suspension (7.83 mg/mL S9, 20.5 mg/g liver)
Cytochrome P450 content	2.52 nmol/mg microsomal protein
Cytochrome b_5 content	0.495 nmol/mg microsomal protein
NADPH-cytochrome-*c* reductase	218 nmol cytochrome *c* reduced /mg microsomal protein/min
Aniline hydroxylase	102 nmol p-aminophenol formed /mg microsomal protein/h

＊1: 21 mL of S9 fraction was centrifuged at 105,000 × *g* for 60 minutes, and 10.5 mL of 1.15 % KCl solution was added to the precipitate. The volume of its suspension was 11.3 mL.

Ames test (50 μL S9 used per plate) *1 :

Mutagen	Dose*2	Strain	His^+ revertant colonies
Benzo(a)pyrene	5.0	TA100	1160 ~ 1169
	5.0	TA98	267 ~ 318
	5.0	TA1537	141 ~ 148
2-Aminoanthracene	1.0	TA100	1304 ~ 1314
	0.5	TA98	476 ~ 506
	2.0	TA1537	208 ~ 244
9,10-Dimethylanthracene	25.0	TA98	195 ~ 196
Spontaneous revertant colonies		TA100	165 ~ 169
		TA98	22 ~ 27
		TA1537	14 ~ 20

＊1 : plate= Minimal agar plate(Oriental Yeast Co.,Ltd.).
Cofactor = Cofactor-I (Oriental Yeast Co.,Ltd.).
＊2 : μg/plate

Note : Products should be stored below −80℃.

Date of assay : November 30, 2016

Expiration date : May 17, 2017

Approved by :

Added page

QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: Reverse Mutagenicity Test of APFHx(C-1500N) on Bacteria

Study Number: K01-6462

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 28, 2017	February 28, 2017
Preparation of test substance solutions and treatment of tester strains	March 2, 2017	March 2, 2017
Observation and colony counting	March 6, 2017	March 6, 2017
Raw data and draft final report	March 24, 2017	March 24, 2017
Final Report	April 3, 2017	April 3, 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
Preparation and management of positive control substance solutions	September 26, 2016	April 3, 2017
Preparation and management of positive control substance solutions	January 10, 2017	April 3, 2017
Preparation and management of positive control substance solutions	February 13, 2017	April 3, 2017
Preparation of medium and reagents	March 10, 2016	April 3, 2017
Management of tester strains	March 28 and April 1,4, 21,22, 2016	April 3, 2017
Pre-cultures	January 16 and 17, 2017	April 3, 2017

Date: April 3, 2017

Quality Assurance Manager: ___

QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: Reverse Mutagenicity Test of APFHx(C-1500N) on Bacteria

Study Number: K01-6462

I confirmed that the correction of final report was conducted correctly. The inspections of correction were carried out and the results were reported to the Study Director and the Test Facility Management as follows.

Item of inspection	Date of inspection	Date of report
Draft amendment 1of final report	April 24, 2017	April 24, 2017
Amendment 1of final report	April 25, 2017	April 25, 2017

This statement was added to the Quality Assurance Statement issued on April 3, 2017.

Date: April 25, 2017

Quality Assurance Manager: _

CERI

AMMENDMENT 1 OF FINAL REPORT

Chemicals Evaluation and Research Institute, Japan, Hita

1. TITLE (STUDY NUMBER)

Reverse Mutagenicity Test of APFHx(C-1500N) on Bacteria (K01-6462)

2. AMENDED ITEM

i) TABLE OF CONTENTS (Page 4)

(The reason and the detail of amendment are shown in annex 1.)

ii) Appendix 2 Assay data of S9 (Page 20)

(The reason and the detail of amendment are shown in annex 2.)

3. APPROVAL BY AUTHOR

Study Director:

April 25, 2017

Date

This document is exact copy of the original.

Date: April 25, 2017

Study director:

Annex 1

Amended item

i) TABLE OF CONTENTS (Page 4)

Reason for amendment

Because the assay data of S9 was added.

Detail of amendment

Before amendment	TABLE OF CONTENTS (syncopation) Appendix 1 Historical data.....19 (syncopation)
After amendment	TABLE OF CONTENTS (syncopation) Appendix 1 Historical data.....19 Appendix 2 Assay data of S9.....20 (syncopation)

Annex 2

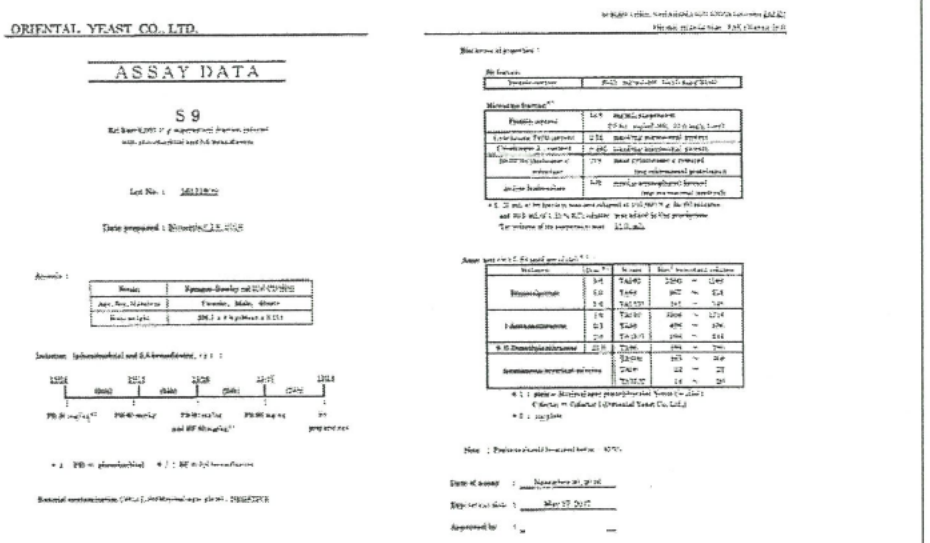
Amended item

ii) Appendix 2　Assay data of S9 (Page 20)

Reason for amendment

Because the assay data of S9 was added.

Detail of amendment

Before amendment	Appendix 2 None
After amendment	Appendix 2　Assay data of S9 

ORIENTAL YEAST CO., LTD.

ASSAY DATA

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QUALITY ASSURANCE STATEMENT