STATEMENT

TITLE OF STUDY

Mutagenicity Test of 13F-EtOH Using Microorganisms (Study Code: K01-3688)

I, the undersigned, hereby declare that this report provides a correct English translation of the final report (Study Code: K01-3688, issued on February 14, 2007) audited by Quality Assurance Unit of CERI Hita, Chemicals Evaluation and Research Institute, Japan.

September 1, 2009

Date

CERI Hita

Chemicals Evaluation and Research Institute, Japan

CERI

STUDY CODE: K01-3688

Receipt No. 836-06-T-5274

FINAL REPORT

MUTAGENICITY TEST OF
13F-EtOH
USING MICROORGANISMS

February 2007

Hita Laboratory
Chemicals Evaluation and Research Institute
Japan

GLP STATEMENT

Hita Laboratory
Chemicals Evaluation and Research Institute, Japan

Sponsor: DAIKIN INDUSTRIES, LTD
Title: Mutagenicity Test of 13F-EtOH Using Microorganisms
Study Code: K01-3688

I, the undersigned, hereby declare that this study was conducted in compliance with "Standards to be observed by Testing Institutions for Toxicity Investigations" (Ministry of Labor, Notification No.76, September 1, 1988; partially revised on March 29, 2000), "Concerning Standard of the Testing Facilities Conducting the Test Relating to the New Chemical Substances" (Notification No. 1121003 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 3 (2003.11.17) of the Manufacturing Industries Bureau, METI & No. 031121004 of the Environmental Health Department, MOE (November 21, 2003)) and "OECD Principles of Good Laboratory Practice" (November 26, 1997).

Management: Signed in original February 14, 2007

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

Study Director: Signed in original February 14, 2007

QUALITY ASSURANCE STATEMENT

Hita Laboratory
Chemicals Evaluation and Research Institute, Japan

Sponsor: DAIKIN INDUSTRIES, LTD
Title: Mutagenicity Test of 13F-EtOH Using Microorganisms
Study Code: K01-3688

This study was audited and inspected by Quality Assurance Section of Hita Laboratory, Chemicals Evaluation and Research Institute, Japan. The dates audited and/or inspected and the dates reported these results to the study director and the management are as follows.

Items of Inspections/Audits	Dates of Inspections/Audits	Dates of Report of Inspections/Audits
Protocol	January 11, 2007	January 11, 2007
Preparation of Test Substance	January 12, 2007	January 12, 2007
Treatment of Test Strains	January 12, 2007	January 12, 2007
Re-inspection of Protocol	January 19, 2007	January 19, 2007
Approval of Protocol	January 23, 2007	January 24, 2007
Raw Data and Draft Final Report	February 11, 2007	February 13, 2007
Re-inspection of Raw Data and Draft Final Report	February 13, 2007	February 13, 2007
Draft Final Report (2nd)	February 13, 2007	February 13, 2007
Re-inspection of Draft Final Report (2nd)	February 13, 2007	February 13, 2007
Final Report	February 14, 2007	February 14, 2007

An item of audit shown below was reported to the study director and the management based on the audit result of institution or another test result.

Item of Audits	Dates of Audits	Date of Report of Audits
Preparation and Management of Positive Control Substances	November 16, 2006	February 14, 2007
Management of Test Strains	October 26, 27, November 9 and 10, 2006	February 14, 2007
Pre-cultures of Test Strains	November 27 and 28, 2006	February 14, 2007
Culture Condition and Observation and Colony Count	October 10 and November 28, 2006	February 14, 2007

I, the undersigned, hereby declare that this study was conducted in compliance with "Standards to be observed by Testing Institutions for Toxicity Investigations" (Ministry of Labor, Notification No.76, September 1, 1988; partially revised on March 29, 2000), "Concerning Standard of the Testing Facilities Conducting the Test Relating to the New Chemical Substances" (Notification No. 1121003 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 3 (2003.11.17) of the Manufacturing Industries Bureau, METI & No. 031121004 of the Environmental Health Department, MOE (November 21, 2003)) and "OECD Principles of Good Laboratory Practice" (November 26, 1997).
This study was also conducted in compliance with "Standards for Toxicity Investigations" (Ministry of Labor, Notification No.77, September 1, 1988 and Notification No.67, June 2, 1997) and "Procedures of Mutagenicity Test Using Microorganisms and Evaluation of Test Results" (Ministry of Labor, Official Notification, February 8, 1999) and "III Mutagenicity test" of "Reverse-Mutation Assay in Bacteria" prescribed in "Testing Methods Relating to the New Chemical Substances" (Notification No. 1121002 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 2 (2003.11.13) of the Manufacturing Industries Bureau, METI & No.031121002 of the Environmental Health Department, MOE (November 21, 2003)).

I, the undersigned, hereby declare that this report provides an accurate description of the methods and procedures used in this study and that the reported results accurately reflect the raw data obtained.

Section Chief, Quality Assurance: Signed in original February 14, 2007

TABLE OF CONTENTS

Study Code: K01-3688
Test Substance Code: HR6854
Sponsor Code: D-0060

TITLE

Mutagenicity Test of 13F-EtOH Using Microorganisms

SPONSOR

DAIKIN INDUSTRIES, LTD
1-1, Nishi-Hitotsuya, Settsu, Osaka 566-8585, Japan

TESTING FACILITY

Hita Laboratory
Chemicals Evaluation and Research Institute, Japan
822, 3-chome, Ishii-machi, Hita, Oita 877-0061, Japan

PURPOSE OF STUDY

The ability of the test substance to induce mutations was investigated by using *Salmonella typhimurium* and *Escherichia coli*.

TESTING METHOD

This study was conducted in accordance with "Standards for Toxicity Investigations" (Ministry of Labor, Notification No.77, September 1, 1988 and Notification No.67, June 2, 1997), and "Procedures of Mutagenicity Test Using Microorganisms and Evaluation of Test Results" (Ministry of Labor, Official Notification, February 8, 1999) and "III Mutagenicity test" of "Reverse-Mutation Assay in Bacteria" prescribed in "Testing Methods Relating to the New Chemical Substances" (Notification No. 1121002 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 2 (2003.11.13) of the Manufacturing Industries Bureau, METI & No.031121002 of the Environmental Health Department, MOE (November 21, 2003).

GLP COMPLIANCE

This study was conducted in compliance with "Standards to be observed by Testing Institutions for Toxicity Investigations" (Ministry of Labor, Notification No.76, September 1, 1988; partially revised on March 29, 2000), "Concerning Standard of the Testing Facilities Conducting the Test Relating to the New Chemical Substances" (Notification No. 1121003 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 3 (2003.11.17) of the Manufacturing Industries Bureau, METI & No. 031121004 of the Environmental Health Department, MOE (November 21, 2003)) and "OECD Principles of Good Laboratory Practice" (November 26, 1997).

PERIOD OF STUDY

Commencement of Study:	January 10, 2007
Initiation of Experiment	
(Initiation of Treatment of Dose Finding Test):	January 12, 2007
Completion of Experiment	
(Completion of Colony Count):	January 31, 2007
Completion of Study:	February 14, 2007

LOCATION AND PERIOD FOR RETENTION OF DATA

Raw data, protocol, letter of test request, questionnaire, final report and other documentation records will be retained in the archives and the test substance will be retained at the test substance storage room of the testing facility for 10 years after the date of the notification specified under Item 1 of Article 57-3 of Industrial Safety & Health Law. Date of the notification will be communicated from the sponsor to the testing facility. They also will be retained for 10 years after the date of the notification specified under Article 4, Paragraph 1 or Paragraph 2, Article 4-2, Paragraph 2, Paragraph 3 or Paragraph 8, Article 5-4, Paragraph 2, Article 24, Paragraph 2 or Article 25-3, Paragraph 2 of Notification on Testing Methods Relating to the New Chemical Substances. The sponsor will communicate the date of the notification to the testing facility. Treatment of data after termination of the retention period will be carried out with the approval of the sponsor.

RETENTION OF ORIGINAL DOCUMENTS

An original and a duplicate of protocol were drawn up. The former will be retained at the testing facility. The latter was sent to the sponsor.

An original final report is drawn up and will be retained at the testing facility. A copy of the original final report that was recognized to be an accurate copy by the study director will be sent to the sponsor.

STUDY DIRECTOR AND NAMES, ASSIGNED SECTIONS AND JOB ASSIGNMENT OF PERSONNEL

Study Director:

Mutagenicity Section, Hita Laboratory

Study Staff:

Mutagenicity Section, Hita Laboratory
(Preparation of the test substance and count of revertant colonies)

Management:

Hita Laboratory

Person in Charge of Storage of Archives:

General Affairs Section, Hita Laboratory

Person in Charge of Test Substance Management:

Analytical Chemistry Section, Hita Laboratory

AUTHOR OF FINAL REPORT

Study Director: Signed in original February 14, 2007

SUMMARY

The ability of 13F-EtOH 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).

As a result, the mutagenicity of the test substance was judged to be negative because the numbers of revertant colonies in the test substance treatment groups were less than two times that in each negative control in all test strains with and without S9 mix. Therefore, it is concluded that 13F-EtOH has no ability to induce mutations under the present test conditions.

MATERIALS AND METHODS

1. TEST SUBSTANCE AND POSITIVE CONTROL SUBSTANCES

1.1 Test Substance (Information Provided by The Sponsor)

1) Name

2-(Perfluorohexyl)ethanol

Other Name: 13F-EtOH

CAS No.: 647-42-7

2) Lot No.

180804

3) Supplier

DAIKIN INDUSTRIES, LTD

4) Structural formula

$HOCH_2CH_2CF_2CF_2CF_2CF_2CF_2CF_3$

(Molecular formula $C_8H_5F_{13}O$)

5) Purity

99.8%

6) Names and concentrations of impurities

Unknown 0.2%

7) Physicochemical properties

Appearance at ordinary temperature:	Colorless transparent liquid
Molecular weight:	364.10
Stability:	—
Melting point	—
Boiling point	78°C (14 mmHg)
Vapor pressure	—
Density	1.678 g/cm^3 (20°C)
Partition coefficient (1-octanol/water)	—
Hydrolysis	—
Solubility	—

Degree of solubility

Water:	<50 mg/mL (measured at testing facility)
DMSO:	≥50 mg/mL (measured at testing facility)
Acetone:	Soluble (soluble at any ratio)
Others	—

8) Storage conditions

Stored in room temperature and dark place (cabinet No. 1 in the test substance storage room, permissible limit of temperature: 10-30°C).

9) Precautions

Gloves, a mask, head cap and lab coat were worn.

1.2 Positive Controls

Name	Manufacturer	Lot No.	Appearance	Purity	Grade
AF-2*1	Wako Pure Chemical Industries, Ltd.	WAP0369	Red-yellow crystalline powder	100.2%	Special grade
NaN_3*2	Wako Pure Chemical Industries, Ltd.	KLN3948	White crystalline powder	99.8%	Special grade
ICR-191*3	Polysciences, Inc.	534652	Yellow crystalline powder	—	—
2AA*4	Wako Pure Chemical Industries, Ltd.	ASM1101	Yellow-green-brown powder	97.4%	—

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

*2: Sodium azide

*3: 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine·2HCl

*4: 2-Aminoanthracene

1) Storage conditions

Stored in a cool and dark place (refrigerator No. 13 in the test substance storage room, permissible limit of temperature: 1-10°C).

2) Precautions

Gloves, a mask, head cap and lab coat were worn.

2. BACTERIAL STRAINS

2.1 Strains and Reason for Selection

Salmonella typhimurium strains TA100, TA1535, TA98 and TA1537 and *Escherichia coli* strain WP2*uvrA* were used. *Salmonella typhimurium* strains and *Escherichia coli* strain were supplied from Dr. Taijiro Matsushima, Japan Bioassay Research Center, on March 13, 2003 and on September 20, 2003, respectively. These test strains have been recommended to use for the mutagenicity test using microorganisms in "Standards for Toxicity Investigations" and "Testing Methods Relating to the New Chemical Substances".

2.2 Storage

Amino acid requirement, sensitivity to ultraviolet-rays, rfa membrane mutation, presence or absence of plasmid pKM101 and negative and positive control values of test strains were examined in the testing facility. Dimethyl sulfoxide (DMSO, Lot No. TY025, ≥99.0% in purity, spectrophotometric grade, DOJINDO Laboratories) was added to fresh overnight cultures of the test strains which had been confirmed to have these properties at a volume ratio of 0.9:10. The mixture was frozen as a stock culture in an ultra-deep freezer (MDF-293AT in the test room No. 2 of the biotron No. 7, SANYO Electric Biomedical Co., Ltd.) below -80°C. The mixture was thawed just before use.

3. MEDIUM AND S9 MIX

3.1 Medium

1) Minimal glucose agar plate

Tesmedia AN (Oriental Yeast Co., Ltd.) was used.

Lot No.: ANIX160KV (manufactured on November 9, 2006, dose finding test)
ANIX340LV (manufactured on December 23, 2006, main test)

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. 6080253, Difco Laboratories) and 0.5 w/v% NaCl at a volume ratio of 1 to 10.

3.2 S9 Mix

1) Rat liver S9

S9 (Lot No. 06090106, manufactured on September 1, 2006, Oriental Yeast Co., Ltd.) prepared from the liver of 7 week-old male SD rats (body weight: 215.1±10.7 g) treated with a combination of phenobarbital and 5, 6-benzoflavone was used. The S9 was cryopreserved in an ultra-deep freezer (MDF-293AT in the test room No. 2 of the biotron No. 7, SANYO Electric Biomedical Co., Ltd.) below -80°C

until use. S9 was thawed just before use.

2) Composition of S9 mix

S9 mix was prepared using Cofactor I® (Lot No. 999603 (dose finding test) and 999604 (main test), Oriental Yeast Co., Ltd.) for S9 mix immediately 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.

4. PRE-CULTURES OF THE TEST STRAINS

Thirty microliters (for TA100), 5-μL (for WP2*uvrA*), and 20-μL (for TA98, TA1535 and TA1537) aliquots of frozen stock culture of bacterial strains were respectively inoculated to 11 mL of 2.5% Nutrient broth No. 2 (Lot No. 298714, OXOID Ltd.) in each L tube (volume: 27 mL). The culture was incubated at 37±0.5°C for 9 hours by shaking at about 50 times/minute in a seesaw type of shaker (MONOSIN-IIA, Taitec Corporation).

The number of viable cells was calculated from O.D. value at 660 nm measured by a spectrophotometry (Novaspec II, Pharmacia Biotech Ltd.) at the end of incubation to calculate the number of viable cells. It was confirmed that the numbers of viable cells were more than 2.3×10^9 cells/mL in *Salmonella typhimurium* and more than 3.8×10^9 cells/mL in *Escherichia coli* strain. When the numbers of viable cells were more than 2.7×10^9 cells/mL in *Salmonella typhimurium* and more than 4.2×10^9 cells/mL in *Escherichia coli* strain, the O.D. value was measured at 660 nm by a spectrophotometry (the same above) and were adjusted with Nutrient broth No. 2 at 2.3-2.7×10^9 cells/mL and at 3.8-4.2×10^9 cells/mL, respectively. The culture was used without adjusting when the *Salmonella typhimurium* strains were in the range of 2.3-2.7×10^9 cells/mL and the *Escherichia coli* strain was in the range of 3.8-4.2×10^9 cells/mL. The final numbers of prepared viable cells is shown below:

Test	TA100	TA1535	WP2*uvrA*	TA98	TA1537
Dose finding test	2.6	2.5	4.0	2.5	2.5
Main test	2.6	2.5	3.8	2.5	2.5

($\times10^9$cells/mL)

5. PREPARATION OF TEST SUBSTANCE AND POSITIVE CONTROL SUBSTANCES

5.1 Preparation of Test Substance

1) Solvent

DMSO (Lot No. TY025 (dose finding test) and TA026 (main test)) was used.

2) Reason for selection of solvent

The test substance was insoluble in 50 mg/mL of distilled water and was soluble in

50 mg/mL of DMSO. The test substance solution of 50 mg/mL prepared with DMSO was considered to be stable from the facts that there was no change in color nor heat generation at room temperature within 2 hours after preparation. Therefore, DMSO was preferably selected as a solvent.

3) Preparation method

DMSO was added to the test substance and mixed by a tube mixer to make a 50 mg/mL of the test substance solution. The test substance solution was diluted with the same solvent to give each required concentration.

4) Preparation time

The test substance solution was prepared immediately before use, kept under yellow lamps at room temperature and used within 2 hours.

5.2 Preparation of Positive Controls

1) Preparation method

NaN_3 was dissolved in distilled water (distilled water for injection, Lot No. K6F74, Otsuka Pharmaceutical Factory Inc.). AF-2, ICR-191 and 2AA were dissolved in DMSO (Lot No. TY025).

2) Storage conditions

Positive control solutions were stored in an ultra-deep freezer (MDF-293AT in the test room No. 2 of the biotron No. 7, SANYO Electric Biomedical Co., Ltd.) below -80°C. The solutions were thawed before use.

6. METHODS

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

6.1 Procedures

After 0.1 mL of the test substance solution, solvent or the positive control solution, 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.

6.2 Sterility

The highest concentration of the test substance suspension (0.1 mL) and S9 mix (0.5 mL) were respectively 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 that plate after 48 hours incubation of 37±0.5°C.

6.3 Negative Control and Positive Controls

The solvent used in the tests was employed as a negative control and the following positive controls were used for each bacterial strain.

	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)

6.4 Dose Selection

1) Dose finding test

A total of 6 doses consisting of 5000 μg/plate as the highest dose and 5 lower doses diluted with a geometric progression of 4 were employed.

2) Main test

The results of the dose finding test showed that the number of revertant colonies in the test substance treatment groups with and without S9 mix was less than twice that in the solvent control. The bacterial growth inhibition was observed at 5000 μg/plate in TA100 and TA98 and more than 1250 μg/plate in TA1535 and TA1537 in the groups of treatment with and without S9 mix. The bacterial growth inhibition was not observed in WP2*uvrA* in the groups of treatment with and without S9 mix. The precipitation of the test substance was not observed in the groups of treatment with and without S9 mix.

Base on the results of the dose finding test, the highest dose was selected at 5000 μg/plate as observed the bacterial growth inhibition and lower 5 doses of 2500, 1250, 625, 313 and 156 μg/plate diluted with a geometric progression of 2, were employed in TA100 and TA98 with and without S9 mix in the main test. The highest dose was selected at 1250 μg/plate as observed the bacterial growth inhibition and lower 5 doses of 625, 313, 156, 78.1 and 39.1 μg/plate diluted with a geometric progression of 2, were employed in TA1535 and TA1537 with and without S9 mix in the main test. The highest dose was selected at 5000 μg/plate and lower 5 doses of 2500, 1250, 625, 313 and 156 μg/plate diluted with a geometric progression of 2, were employed in WP2*uvrA* with and without S9 mix

in the main test.

7. OBSERVATION AND COLONY COUNTING

7.1 Observation

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

7.2 Colony Counting

For the plates in which the bacterial growth inhibition was observed, the number of colonies was counted with a manual counter, and the other plates were counted by using a colony analyzer (CA-11D, System Science Ltd.). Square correction and miss counting correction were performed in colony analyzer.

8. JUDGEMENT CRITERIA OF TEST RESULTS

The test substance was judged to be positive when the number of revertant colonies increased to twice or more that in the negative control in a concentration-dependent manner and also the reproducibility of the test results was obtained. In all other cases, it was judged to be negative. Any statistical methods were not used.

FACTORS AFFECTED RELIABILITY OF TEST

There were no factors that might have affected the reliability of the test.

TEST RESULTS

1. DOSE FINDING TEST

The test result and the dose-response curves are shown in Table 1, Fig. 1 and Fig. 2.

The number of revertant colonies in the test substance treatment groups with and without S9 mix was less than twice that in the solvent control. The bacterial growth inhibition was observed at 5000 μg/plate in TA100 and TA98 and more than 1250 μg/plate in TA1535 and TA1537 in the groups of treatment with and without S9 mix. The bacterial growth inhibition was not observed in WP2*uvrA* in the groups of treatment with and without S9 mix. The precipitation of the test substance was not observed in the groups of treatment with and without S9 mix.

2. MAIN TEST

The test result and the dose-response curves are shown in Table 2, Fig. 3 and Fig. 4.

The number of revertant colonies in the test substance treatment groups with and without S9 mix was less than twice that in the solvent control. The bacterial growth

inhibition was observed more than 2500 μg/plate in TA100 and TA98 and more than 625 μg/plate in TA1535 and TA1537 in the groups of treatment with and without S9 mix. The bacterial growth inhibition was not observed in WP2*uvrA* in the groups of treatment with and without S9 mix. The precipitation of the test substance was not observed in the groups of treatment with and without S9 mix.

DISCUSSION AND CONCLUSION

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

The numbers of the revertant colonies in the positive controls were above two times that in the negative controls. The test results showed that the numbers of revertant colonies in the negative control and the 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 indicates the test results to be valid.

From the above results, it was concluded that 13F-EtOH had no ability to induce mutations under the present test conditions.

Table 1 Results of the dose finding test

Test substance: 13F-EtOH

Test Period		From January 12, 2007 to January 15, 2007				
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	104 118 111 (111)	7 12 10 (10)	37 34 45 (39)	28 28 22 (26)	21 16 16 (18)
	4.88	135 116 (126)	5 12 (9)	48 40 (44)	31 35 (33)	21 16 (19)
	19.5	120 124 (122)	13 18 (16)	33 47 (40)	30 22 (26)	23 10 (17)
	78.1	110 99 (105)	12 7 (10)	36 34 (35)	19 23 (21)	10 7 (9)
	313	111 115 (113)	11 5 (8)	43 36 (40)	20 20 (20)	16 12 (14)
	1250	94 99 (97)	7* 3* (5)	26 34 (30)	19 13 (16)	3* 3* (3)
	5000	88* 93* (91)	5* 2* (4)	34 30 (32)	12* 24* (18)	4* 6* (5)
+S9 mix	Negative control	135 115 121 (124)	11 16 10 (12)	34 47 56 (46)	42 37 33 (37)	35 35 37 (36)
	4.88	157 135 (146)	6 11 (9)	52 57 (55)	41 47 (44)	41 42 (42)
	19.5	146 138 (142)	7 10 (9)	51 55 (53)	38 31 (35)	50 45 (48)
	78.1	123 114 (119)	14 18 (16)	47 50 (49)	23 38 (31)	25 30 (28)
	313	101 104 (103)	12 14 (13)	56 56 (56)	38 33 (36)	29 19 (24)
	1250	109 88 (99)	5* 9* (7)	41 66 (54)	31 38 (35)	8* 9* (9)
	5000	98* 99* (99)	4* 11* (8)	48 40 (44)	25* 28* (27)	13* 20* (17)
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	711 668 (690)	447 460 (454)	413 401 (407)	593 577 (585)	1211 1318 (1265)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	1288 1211 (1250)	284 220 (252)	454 446 (450)	438 415 (427)	327 239 (283)

[Notes]

- ( ): The mean of each plate.
- *: Bacterial growth inhibition was observed.
- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- ICR-191: 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine·2HCl
- 2AA: 2-Aminoanthracene

Table 2 Results of the main test

Test substance: 13F-EtOH

Test Period		From January 29, 2007 to January 31, 2007				
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	145 130 156 (144)	14 16 12 (14)	28 27 25 (27)	28 38 26 (31)	8 12 12 (11)
	39.1	—	13 7 (10)	—	—	11 4 (8)
	78.1	—	7 11 (9)	—	—	13 8 (11)
	156	108 126 (117)	13 7 (10)	29 31 (30)	31 33 (32)	7 8 (8)
	313	129 117 (123)	13 8 (11)	18 25 (22)	28 20 (24)	4 11 (8)
	625	126 123 (125)	9* 10* (10)	18 29 (24)	21 16 (19)	11* 5* (8)
	1250	121 105 (113)	8* 8* (8)	25 26 (26)	18 15 (17)	8* 9* (9)
	2500	112* 110* (111)	—	31 19 (25)	17* 25* (21)	—
	5000	136* 106* (121)	—	38 22 (30)	21* 21* (21)	—
+S9 mix	Negative control	146 132 145 (141)	12 12 8 (11)	26 37 22 (28)	38 50 47 (45)	23 35 30 (29)
	39.1	—	12 11 (12)	—	—	28 31 (30)
	78.1	—	11 17 (14)	—	—	36 29 (33)
	156	89 112 (101)	13 12 (13)	35 35 (35)	29 30 (30)	19 12 (16)
	313	115 117 (116)	11 12 (12)	35 29 (32)	45 35 (40)	18 20 (19)
	625	114 114 (114)	12* 7* (10)	27 22 (25)	21 31 (26)	17* 17* (17)
	1250	91 117 (104)	5* 5* (5)	25 40 (33)	33 37 (35)	15* 11* (13)
	2500	109* 104* (107)	—	38 29 (34)	22* 29* (26)	—
	5000	111* 124* (118)	—	31 23 (27)	38* 31* (35)	—
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	674 719 (697)	517 574 (546)	346 353 (350)	694 629 (662)	1779 1682 (1731)
Positive control +S9 mix	Chemical	2AA	2AA	2AA	2AA	2AA
	Dose(μg/plate)	1	2	10	0.5	2
	Number of revertant colonies/plate	1158 1034 (1096)	271 277 (274)	573 548 (561)	372 380 (376)	241 275 (258)

[Notes]

- ( ): The mean of each plate.
- *: Bacterial growth inhibition was observed.
- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- ICR-191: 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine·2HCl
- 2AA: 2-Aminoanthracene

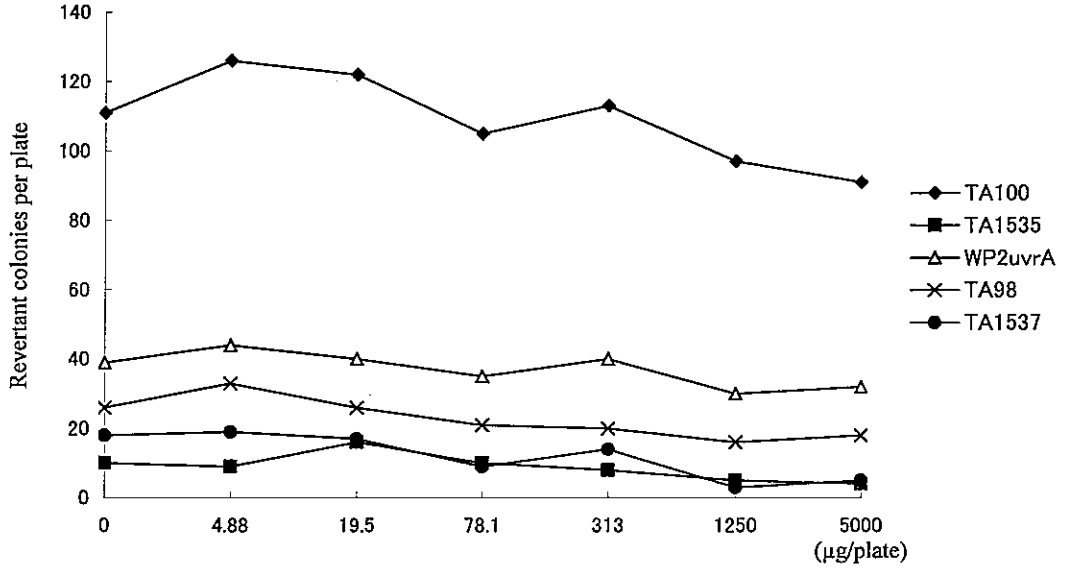

Fig. 1 Dose-response curve without S9 mix in the dose finding test

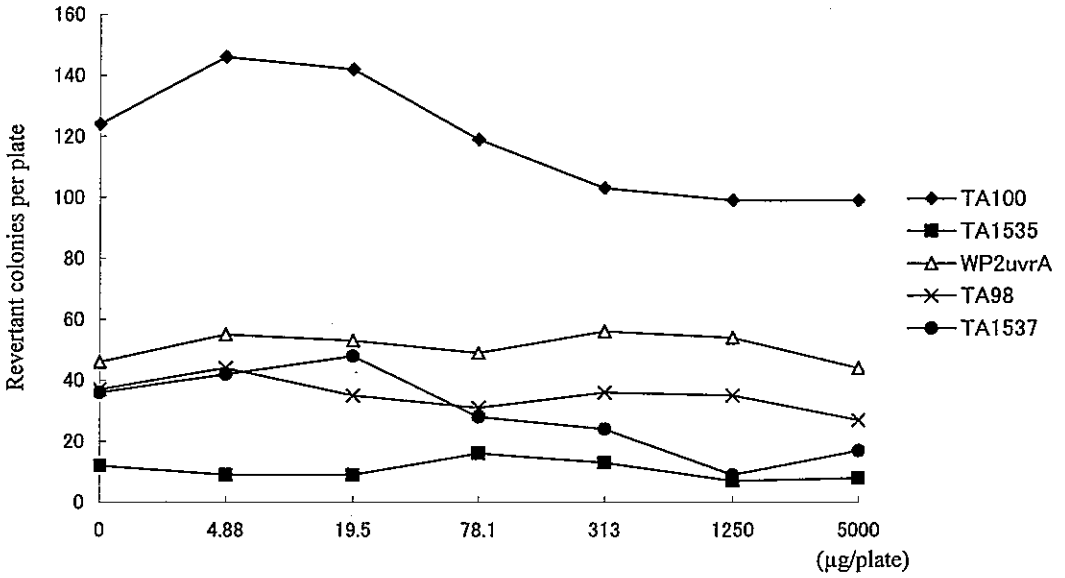

Fig. 2 Dose-response curve with S9 mix in the dose finding test

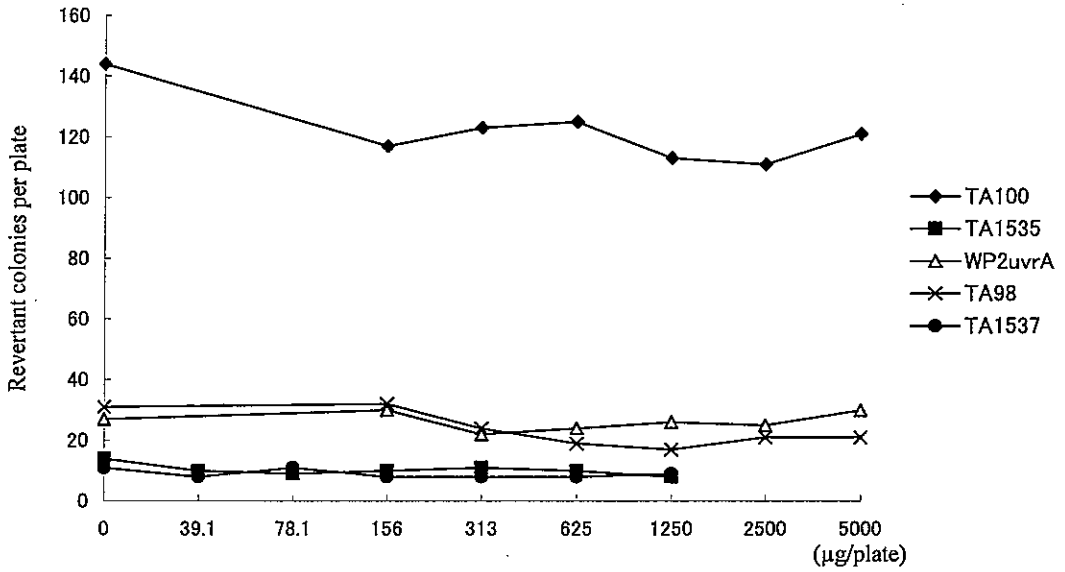

Fig. 3 Dose-response curve without S9 mix in the main test

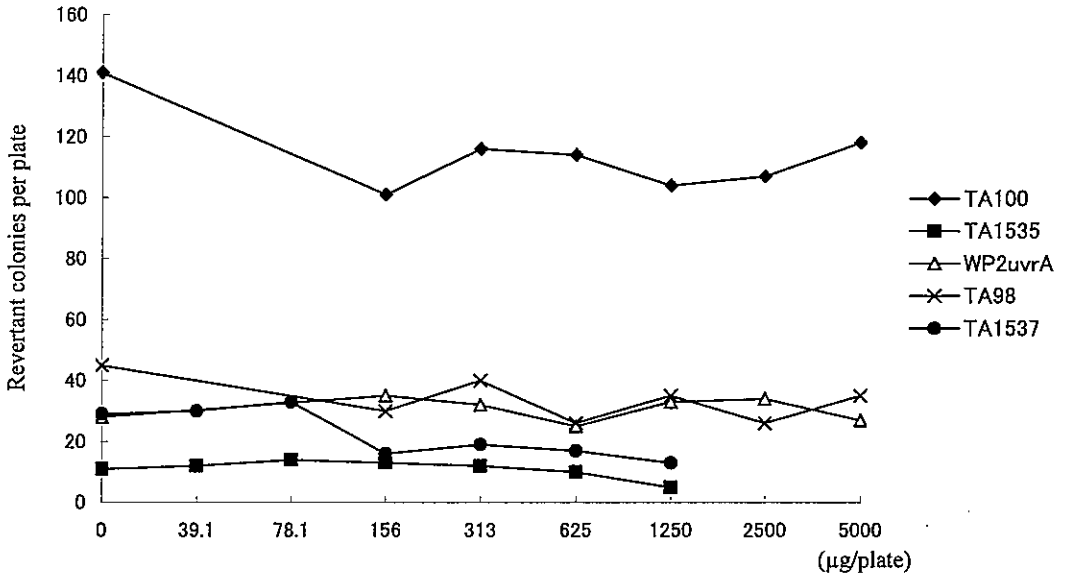

Fig. 4 Dose-response curve with S9 mix in the main test

HISTORICAL DATA (K01-3688)
(Tesmedia AN)

July, 2006 -December, 2006

Negative Control (Mean±3S.D.)

	-S9 mix					+S9 mix				
	TA100	TA1535	WP2*uvrA*	TA98	TA1537	TA100	TA1535	WP2*uvrA*	TA98	TA1537
Mean	124	12	26	23	10	130	10	30	33	23
S.D.	17	4	6	5	3	19	3	7	6	7
Upper Limit	175	24	44	38	19	187	19	51	51	44
Lower Limit	73	1	8	8	1	73	1	9	15	2

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	702	464	389	575	1853	1122	243	525	424	237
S.D.	74	63	58	47	251	138	28	106	52	35
Upper Limit	924	653	563	716	2606	1536	327	843	580	342
Lower Limit	480	275	215	434	1100	708	159	207	268	132

[Notes]
- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- ICR-191: 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine·2HCl
- 2AA: 2-Aminoanthracene