USA-R-06397

FINAL REPORT

Mutagenicity Test of C6 Methacrylate (M-1620) by using Microorganisms

August 4, 2006

UBE SCIENTIFIC ANALYSIS LABORATORY, INC.

Certificate of Translation

Title: Mutagenicity Test of C6 Methacrylate (M-1620) by using Microorganisms

Sponsor: Daikin Industries, Ltd.

Study code number: USA-R-06397

I hereby certify that this translated report accurately reflects the original final report of the above study.

Organizational assignment UBE SCIENTIFIC ANALYSIS LABORATORY, INC.

Address 1978-6, Aza-okinoyama Ooaza-kogushi, Ube-city,
Yamaguchi-pref., Japan

Name Junichi Hashimoto
Junichi Hashimoto

Date December 19, 2011

GLP STATEMENT

UBE Scientific Analysis Laboratory, Inc.

Sponsor : Daikin Industries, Ltd.
Title : Mutagenicity Test of C6 Methacrylate (M-1620) by using Microorganisms
Study code number: USA-R-06397

This test was conducted according to the Joint Notification Yakusyoku No.1121003 of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, H15・11・17 seikyoku No.3 of the Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, kanpoki No.031121004 of the Environmental Policy Bureau, Ministry of the Environment (November 21, 2003) and the Joint Notification Yakusyoku No.1121002 of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, H15・11・13 seikyoku No.2 of the Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, kanpoki No.031121002 of the Environmental Policy Bureau, Ministry of the Environment (November 21, 2003) and the Notification of Ministry of Labour, No.76, September 1, 1988 and No.13 (revised), March 29, 2000 and the Notification of Ministry of Labour, No.77, September 1, 1988 and No.67 (revised), June 2, 1997.

I, the undersigned, hereby declare that this report provides an accurate and faithful record of the results obtained.

Study Director _____ August 4, 2006
Yukihiro Noguchi

Quality Assurance Certificate

1. Kind of test

Mutagenicity Test by using Microorganisms
(study code No. USA-R-06397)

2.Name of the test substance

C6 Methacrylate (M-1620)

I certify that the methods and procedures used in the test are described precisely in the final report, that test was precisely performed according to the protocol and standards of procedure, and that the reported results reflect the raw-data accurately.

The circumstances of audits and inspections are as follows.

Account

Date Conducted of audits or inspections	Object	Date Reported to the Study Director	Date Reported to the Administrator
July 10, 2006	Protocol	July 10, 2006	July 10, 2006
July 11, 2006	Circumstances of performance of the test (Test Procedure)	July 11, 2006	July 11, 2006
July 13, 2006	Circumstances of performance of the test (Plate Observation)	July 13, 2006	July 13, 2006
August 3, 2006	Revise of Protocol	August 3, 2006	August 3, 2006
August 4, 2006	Final Report (draft)	August 4, 2006	August 4, 2006
August 4, 2006	Final Report	August 4, 2006	August 4, 2006

Individual responsible for quality assurance

Organizational assignment UBE SCIENTIFIC ANALYSIS LABORATORY, INC.

Title Quality Assurance Unit

Name Tohru Ogawa

Date August 4, 2006

CONTENTS

SUMMARY

This study was designed to assess the mutagenic potential of C6 Methacrylate (M-1620) using a bacterial/microsome test system.

Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2*uvrA* were treated with the test material using the pre-incubation method at six dose levels, in duplicate, both with and without the addition of a rat liver homogenate metabolizing system (10% liver S9 in standard co-factors). The dose range for the dose-determination test was 4.88 to 5000 μg/plate. The experiment was repeated on a separate day using the dose range, 156 to 5000 μg/plate, fresh cultures of the bacterial strains and fresh test material formulations.

Neither cytotoxicity nor precipitate was observed at any dose level with or without metabolic activation.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, at any dose level either with or without metabolic activation.

The test material was considered to be non-mutagenic under the conditions of this test.

1. TITLE

Mutagenicity Test of C6 Methacrylate (M-1620) by using Microorganisms

2. SPONSOR

Daikin Industries, Ltd.
1-1 Nishihitotsuya, Settsu-shi, Osaka, Japan

3. TESTING FACILITY

UBE Scientific Analysis Laboratory, Inc.
1978-6, Aza-Okinoyama, Oaza-Kogushi, Ube-shi, Yamaguchi Prefecture, Japan

4. PURPOSE OF TEST

Purpose of this test is to evaluate the mutagenicity of C6 Methacrylate (M-1620) by the microbial mutagenicity test using *Salmonella typhimurium* and *Escherichia coli*.

5. TESTING METHOD

This test was conducted according to the Joint Notification Yakusyoku No.1121002 of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, H15・11・13 seikyoku No.2 of the Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, kanpoki No.031121002 of the Environmental Policy Bureau, Ministry of the Environment (November 21, 2003) and the Notification of Ministry of the Labour, No.77, September 1, 1988 and No.67 (revised), June 2, 1997.

6. GLP COMPLIANCE

This test was conducted according to the Joint Notification Yakusyoku No.1121003 of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, H15・11・17 seikyoku No.3 of the Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, kanpoki No.031121004 of the Environmental Policy Bureau, Ministry of the Environment (November 21, 2003) and the Notification of Ministry of the Labour, No.76, September 1, 1988 and No.13 (revised), March 29, 2000.

7. PERIOD OF STUDY

Commencement of Study:	July 10, 2006
Initiation of Dose-determination Test:	July 11, 2006
Initiation of Mutagenicity Test:	July 14, 2006
Completion of Study:	August 4, 2006

8. ARCHIVES

The study protocol, raw data, recorded documents, final report, documents pertaining to quality assurance, test material, and other study-related documents will be retained in the archives according to the standard operation procedure of UBE Scientific Analysis Laboratory Inc. for 10 years after receiving the notification under Article 4, Section 1 or Section 2, Article 4-2, Section 2, 3 or 8, Article 5-4, Section 2, Article 24, Section 2, or Article 25-3, Section 2 of Act on the Evaluation of Chemical Substances and Regulation of Their Manufacturing, etc., or for 10 years after submission under Article 57-3, Section 1 of Industrial Safety and Health Law, whichever is longer (the specific storage period is to be decided 10 years after the study completion upon deliberation with the sponsor).

9. AUTHOR OF FINAL REPORT AND PERSONS CONCERNED WITH TEST

Study Director: _____ August 4, 2006

Yukihiro Noguchi

Person in charge of Storage: Masatoshi Iwamoto

Personnel in concerned: Junichi Hashimoto

Mitsuo Takahashi

Ritsuko Tanaka

10. MATERIALS AND METHODS

10.1 Test Material

Name of the new chemical substance	: 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl methacrylate
Other name	: C6 Methacrylate (M-1620)
Lot No.	: 60428
Purity of the new chemical substance tested	: 99.8%
Name and concentration of impurities	: 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctan-1-ol 0.02%
CAS No.	: 2144-53-8
Molecular weight	: 432.17
Boiling point	: 92°C/8 mmHg
Appearance at ordinary temperature	: Colorless transparent liquid
Stability	: Stable at room temperature under light shielding
Degree of solubility	: Water*; <50 g/L : DMSO*; <100 g/L : Acetone*; >100 g/L

* Test result at UBE Scientific Analysis Laboratory

10.2 Tester Strains

Salmonella typhimurium TA100, TA1535, TA98 and TA1537
Escherichia coli WP2*uvrA*

All of the strains were obtained from Dr. T. Matsushima, Japan Bioassay Research Center, Japan Industrial Safety And Association, Hadano-shi, Kanagawa. All of the strains were stored at -80°C. Prior to the master strains being used, characterization checks were carried out to confirm the amino-acid requirement, presence of rfa, R factor, *uvrB* or *uvrA* mutation and the spontaneous reversion rate.

In this assay, overnight sub-cultures of the appropriate coded stock cultures were prepared in nutrient broth and incubated at 37°C for 10 hours. Each culture was monitored spectrophotometrically for turbidity determined by viable count analysis on nutrient agar plates.

10.3 Preparation of Test and Control Materials

Test Material:
The test material was not soluble in water at 50 mg/ml and in DMSO at 100 mg/ml, but was soluble in acetone at 100 mg/ml in solubility checks performed in-house. Acetone was therefore selected as the vehicle of choice. The test material was dissolved in acetone to make a stock solution of 100 mg/ml and further diluted to obtain desired concentrations.

Positive control Materials:

A solvent treatment group was used as the vehicle control and the positive control materials were as follows:

2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2, Wako Pure Chemical, Lot # PKE1831):
AF-2 at 0.1 μg/50 μl/plate for TA98
AF-2 at 0.01 μg/50 μl/plate for TA100 and WP2*uvrA*
Sodium azide (NaN_3, Wako Pure Chemical, Lot # SDP7996): 0.5 μg/50 μl/plate for TA1535
9-Aminoacridine (9-AA, MERCK, Lot # S03761): 80 μg/50 μl/plate for TA1537

In addition, 2-Aminoanthracene (2-AA, Wako Pure Chemical, Lot # KLH1058), which is non-mutagenic in the absence of metabolizing enzymes, was used in the series of plates with S9 mix at the following concentrations:

2-AA at 0.5 μg/50 μl/plate for TA98
2-AA at 1.0 μg/50 μl/plate for TA100
2-AA at 2.0 μg/50 μl/plate for TA1535 and TA1537
2-AA at 10 μg/50 μl/plate for WP2*uvrA*

10.4 Microsomal Enzyme Fraction

S9 was purchased from Oriental Yeast Co., Ltd. S9 (Lot No.06051202) was prepared on May 12, 2006 from the livers of male Sprague-Dawley rats weighing 217.5 ± 10.9 g (Mean ± S.D.). These had each intraperitoneally injected phenobarbital (PB, 4 times 0.03-0.06 g/kg/day) and 5,6-benzoflavone (BF, 1 time 0.08 g/kg/day) prior to S9 separation. The S9 was stored at -80°C.

10.5 S9 mix and Agar

The S9 mix was prepared immediately before use using sterilized co-factors and maintained on ice for the duration of the test.

Constituents	Amount in 1 ml S9 mix
S9	0.1 ml
$MgCl_2$	8 μmol
KCl	33 μmol
Glucose-6-phosphate	5 μmol
NADPH	4 μmol
NADH	4 μmol
Na-phosphate Buffer (pH 7.4)	100 μmol

A 0.5 ml aliquot of S9 mix and 2 ml of molten, trace histidine and biotin or tryptophan supplemented, top agar were overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9 mix. This procedure was repeated on the day of each experiment.

Top agar was prepared using 0.6% Difco Bacto agar and 0.5% sodium chloride with 10 ml of 0.5 mM histidine and 0.5 mM biotin or 0.5 mM tryptophan solution added to each 100 ml of top agar. Vogel-Bonner Minimal agar plates were purchased from Oriental Yeast Co., Ltd.

10.6 Test Procedure

10.6.1 Dose-Determination test

Six concentrations of the test material (4.88, 19.5, 78.1, 313, 1250 and 5000 μg/plate) were assayed in duplicate for each tester strain, using the pre-incubation method.

Measured aliquots of the test material formulation, vehicle or positive control (0.05 ml) were dispensed into sets of test tubes followed by either 0.5 ml of S9 mix or phosphate buffer, 0.1 ml of one of the bacterial cultures. The contents of each test tube were incubated at 37°C for 20 min. and mixed with 2.0 ml of molten, trace histidine and biotin or tryptophan supplemented, top agar and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (one tube per plate). This procedure was repeated, in duplicate, for each bacterial strain and for each concentration of test material both with and without S9 mix.

After 48 hours incubation at 37°C, all of the plates were assessed for numbers of revertant colonies using a colony analyzer CA-11S (System Science Co., Ltd.).

10.6.2 Mutagenicity test

The second experiment was performed using methodology as described for the dose-determination test, using fresh bacterial cultures, test material and control solutions. The test material dose range was between 156 and 5000 μg/plate with or without metabolic activation.

10.7 Evaluation Criteria

The test material may be considered positive in this test system if the following criteria are met:

The test material should have induced a reproducible and dose-dependent increase in the number of revertant colonies to at least twice as many as that of the negative control.

11. RESULTS AND DISCUSSION

The individual plate counts, the mean number of revertant colonies for the test material, vehicle and positive controls both with and without metabolic activation, are presented in Appendix 1 and Appendix 2.

Neither cytotoxicity nor precipitate was observed at any dose level with or without metabolic activation in the dose-determination test and the mutagenicity test.

No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains at any dose level, either with or without metabolic activation.

The test material was considered to be non-mutagenic under the conditions of this test.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9 mix and the sensitivity of the bacterial strains.

12. REFERENCES

[1] Ames, B. N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Res.*, **31**, 347-364.

[2] Matsushima, T., Sawamura, M., Hara, K. and Sugimura, T. A safety substitute for polychlorinated biphenyls as an inducer of metabolic activation system. In: de Serres, F. J., Fouts., J. R., Bend, J.R. and Philpot, R. M. (Eds), (1976). *In vitro Metabolic Activation in Mutagenesis Testing*, Elsevier, Amsterdam, pp. 85-88

[3] Maron, D. and Ames, B. N. (1983) Revised methods for the Salmonella mutagenicity test. *Mutation Res.*, **113**, 173-215.

Appendix 1

Test Results (Dose-determination test)

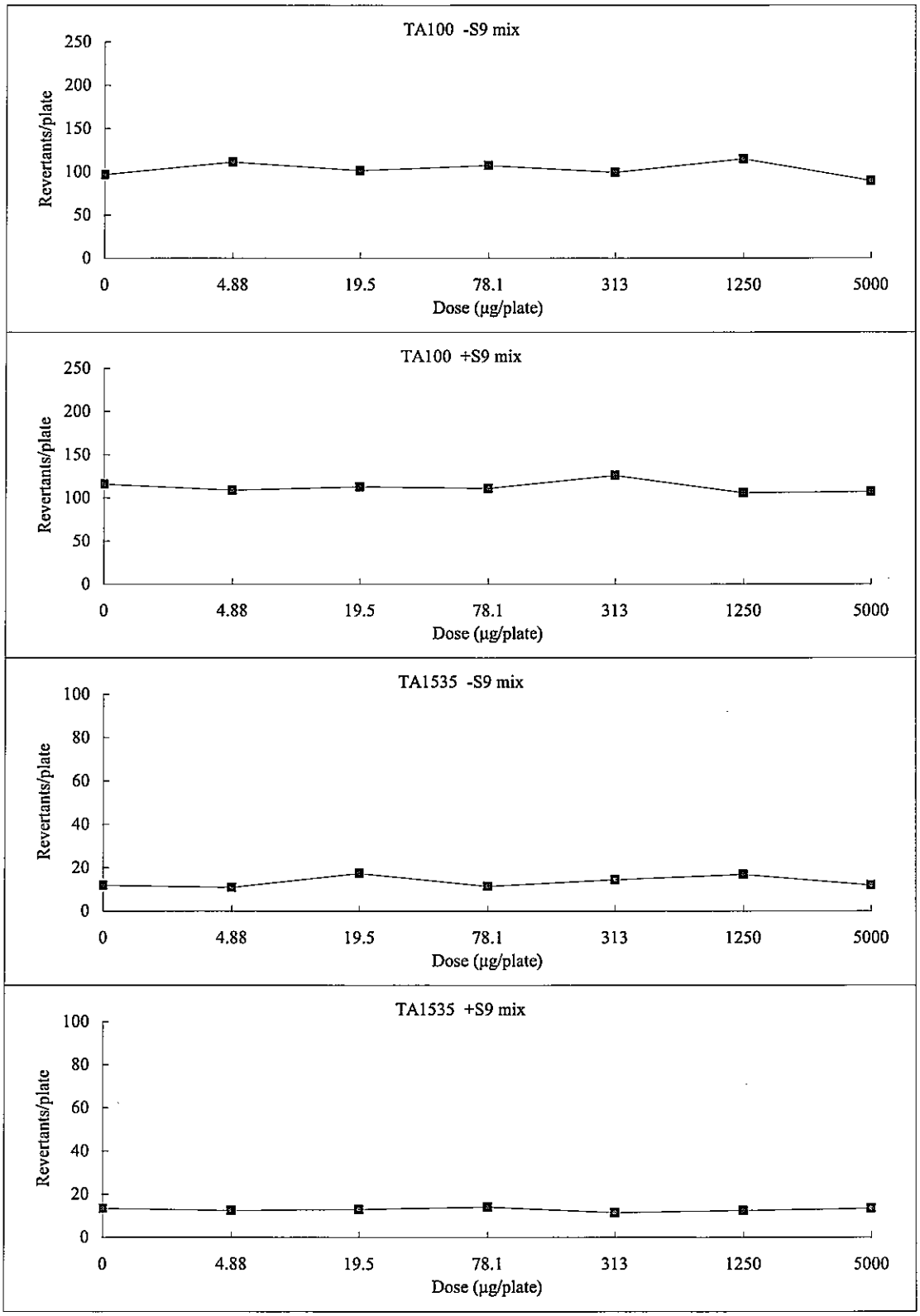
Name of Test Substance: C6 Methacrylate (M-1620)

Test period		From July 11, 2006 to July 13, 2006				
With(+) or Without(-) S9 mix	Test substance concentration (μg/plate)	Number of revertants (Number of colonies/plate)				
		Base-pair substitution type			Frame-shift type	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
-S9 mix	Solvent control	95 (97) 99	11 (12) 13	27 (29) 31	15 (15) 14	5 (5) 4
	4.88	114 (112) 109	14 (11) 8	20 (21) 22	14 (14) 13	4 (5) 5
	19.5	85 (102) 118	17 (18) 18	25 (24) 23	13 (12) 11	8 (8) 7
	78.1	102 (108) 113	13 (12) 10	28 (32) 35	18 (20) 21	3 (6) 8
	313	86 (100) 113	14 (15) 15	24 (22) 20	18 (15) 12	9 (9) 9
	1250	121 (115) 109	17 (17) 17	28 (26) 23	10 (12) 13	4 (7) 9
	5000	92 (90) 87	14 (12) 10	40 (33) 25	18 (19) 20	3 (6) 9
+S9 mix	Solvent control	109 (116) 123	13 (14) 14	33 (27) 21	20 (20) 19	11 (11) 11
	4.88	98 (109) 120	14 (13) 11	29 (25) 21	25 (22) 19	8 (10) 11
	19.5	112 (113) 114	13 (13) 13	28 (32) 35	24 (24) 23	13 (13) 13
	78.1	106 (111) 116	13 (14) 15	23 (29) 35	20 (20) 20	7 (8) 8
	313	109 (126) 143	13 (12) 10	36 (35) 34	23 (21) 18	13 (12) 10
	1250	114 (106) 98	10 (13) 15	28 (28) 28	26 (28) 29	7 (10) 13
	5000	96 (107) 118	15 (14) 12	34 (29) 23	18 (18) 18	10 (9) 8
Positive control not requiring S9 mix	Name	AF-2 [1]	NaN_3 [2]	AF-2	AF-2	9-AA [3]
	concentration (μg/plate)	0.01	0.5	0.01	0.1	80.0
	Number of colonies/plate	672 (643) 614	324 (314) 303	249 (258) 267	526 (512) 498	344 (335) 325
Positive control requiring S9 mix	Name	2-AA [4]	2-AA	2-AA	2-AA	2-AA
	concentration (μg/plate)	1.0	2.0	10.0	0.5	2.0
	Number of colonies/plate	1287 (1255) 1223	296 (289) 281	689 (709) 728	509 (541) 573	229 (238) 246

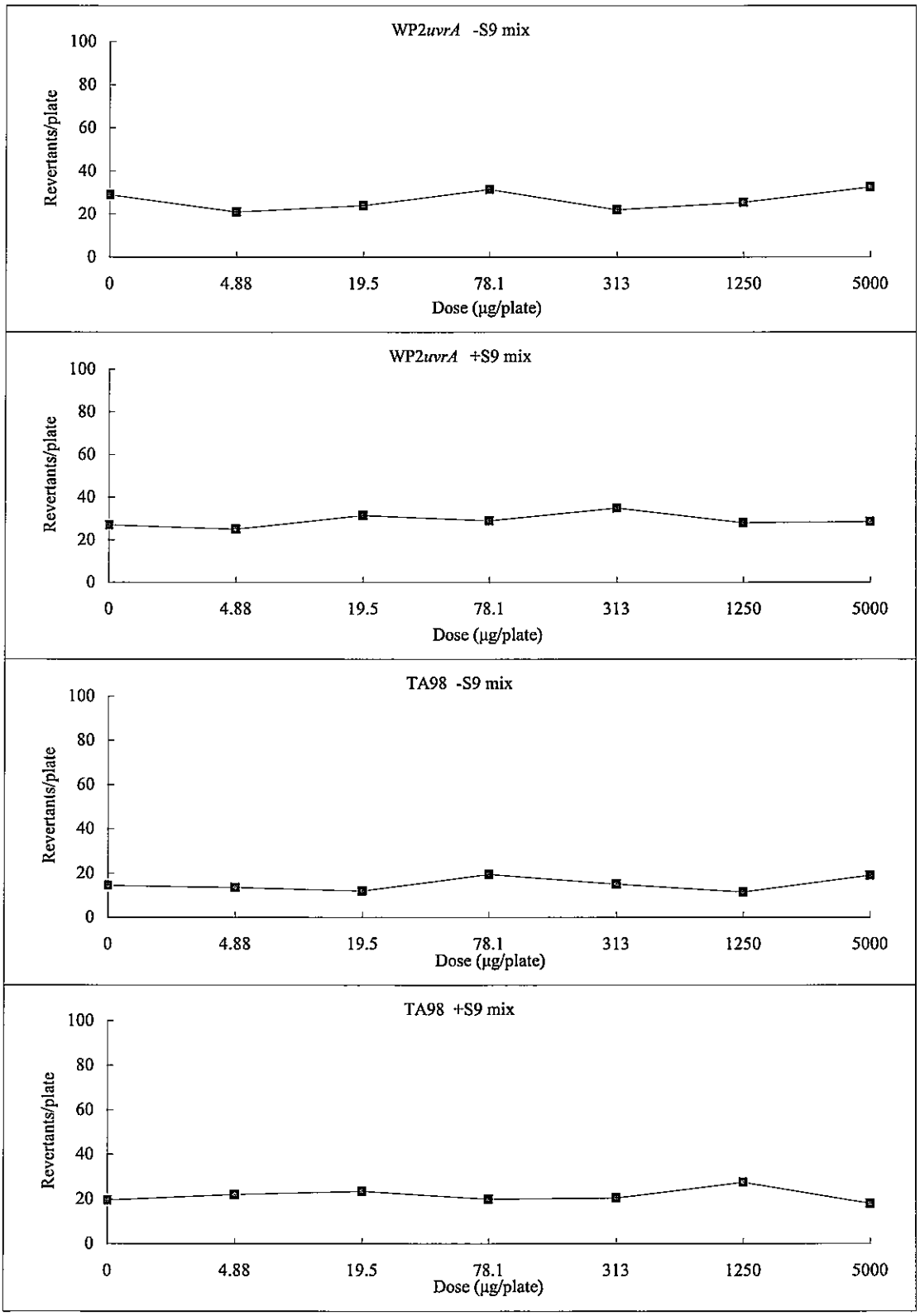
1) AF-2 :2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
2) NaN_3 :Sodiumazide
3) 9-AA :9-Aminoacridine
4) 2-AA :2-Aminoanthracene

[Notes]
1. When toxicity was observed, "*" was placed to the right of the number of the revertants.
2. When precipitation was observed, "†" was placed to the right of the test substance concentration.
3. The average number of revertants in each concentration was showed in ( ).

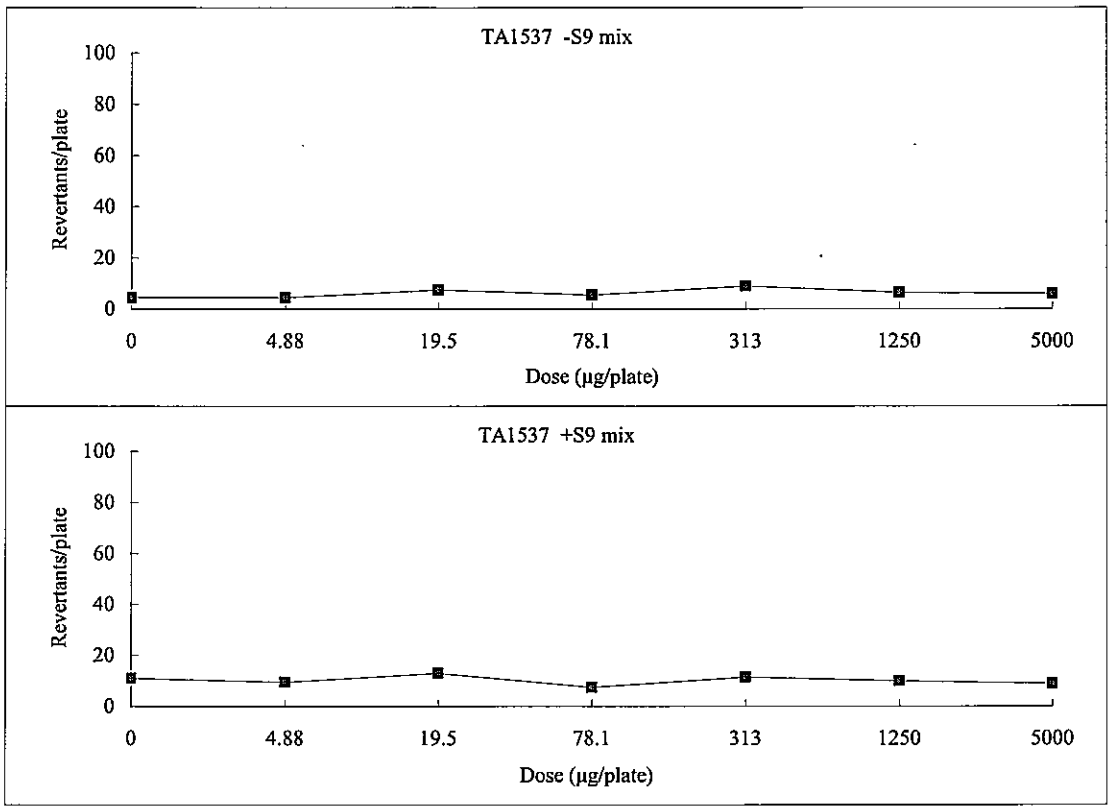
Dose-response curves (Dose-determination test)



Dose-response curves (Dose-determination test)



Dose-response curves (Dose-determination test)



Appendix 2

Test Results (Mutagenicity test)

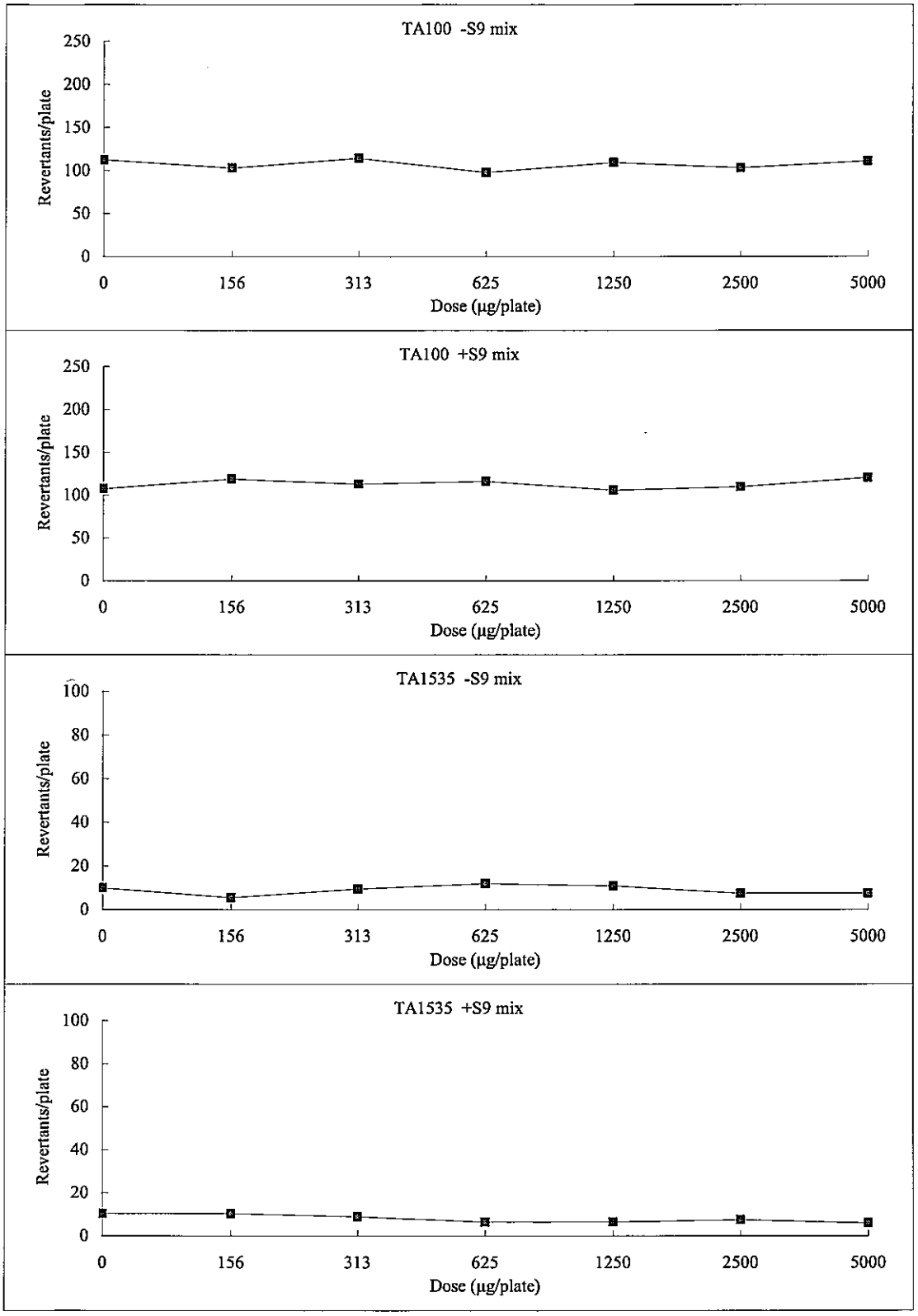
Name of Test Substance: C6 Methacrylate (M-1620)

Test period		From July 14, 2006 to July 18, 2006									
With(+) or Without(-) S9 mix	Test substance concentration (μg/plate)	Number of revertants (Number of colonies/plate)									
		Base-pair substitution type						Frame-shift type			
		TA100		TA1535		WP2*uvrA*		TA98		TA1537	
-S9 mix	Solvent control	111	(113)	10	(10)	28	(29)	14	(17)	11	(8)
		114		10		30		19		5	
	156	103	(103)	3	(6)	20	(21)	16	(13)	7	(6)
		103		8		22		10		5	
	313	112	(115)	11	(10)	34	(35)	20	(18)	5	(8)
		117		8		35		15		10	
	625	105	(98)	10	(12)	29	(31)	10	(10)	8	(8)
		91		14		33		9		8	
	1250	108	(110)	9	(11)	30	(31)	10	(11)	5	(5)
		111		13		32		11		5	
	2500	108	(103)	5	(8)	36	(34)	9	(12)	5	(8)
		98		10		32		14		10	
	5000	118	(111)	7	(8)	39	(36)	14	(14)	5	(6)
		104		8		33		14		7	
+S9 mix	Solvent control	106	(108)	11	(11)	28	(29)	21	(19)	10	(12)
		109		10		29		16		13	
	156	130	(119)	10	(11)	41	(38)	23	(25)	7	(10)
		108		11		35		26		13	
	313	112	(113)	8	(9)	34	(36)	19	(20)	9	(9)
		114		10		37		20		8	
	625	108	(116)	5	(7)	32	(30)	26	(23)	8	(11)
		124		8		28		20		13	
	1250	100	(106)	4	(7)	32	(37)	23	(22)	9	(8)
		112		9		41		20		7	
	2500	112	(110)	10	(8)	25	(31)	16	(18)	14	(13)
		107		5		37		20		11	
	5000	119	(121)	5	(6)	29	(35)	23	(21)	13	(11)
		122		7		41		18		8	
Positive control not requiring S9 mix	Name	AF-2 [1]		NaN_3 [2]		AF-2		AF-2		9-AA [3]	
	concentration (μg/plate)	0.01		0.5		0.01		0.1		80.0	
	Number of colonies/plate	683	(676)	290	(300)	273	(268)	482	(475)	274	(261)
		669		310		263		467		247	
Positive control requiring S9 mix	Name	2-AA [4]		2-AA		2-AA		2-AA		2-AA	
	concentration (μg/plate)	1.0		2.0		10.0		0.5		2.0	
	Number of colonies/plate	1177	(1150)	262	(267)	674	(670)	493	(541)	177	(173)
		1123		271		666		588		168	

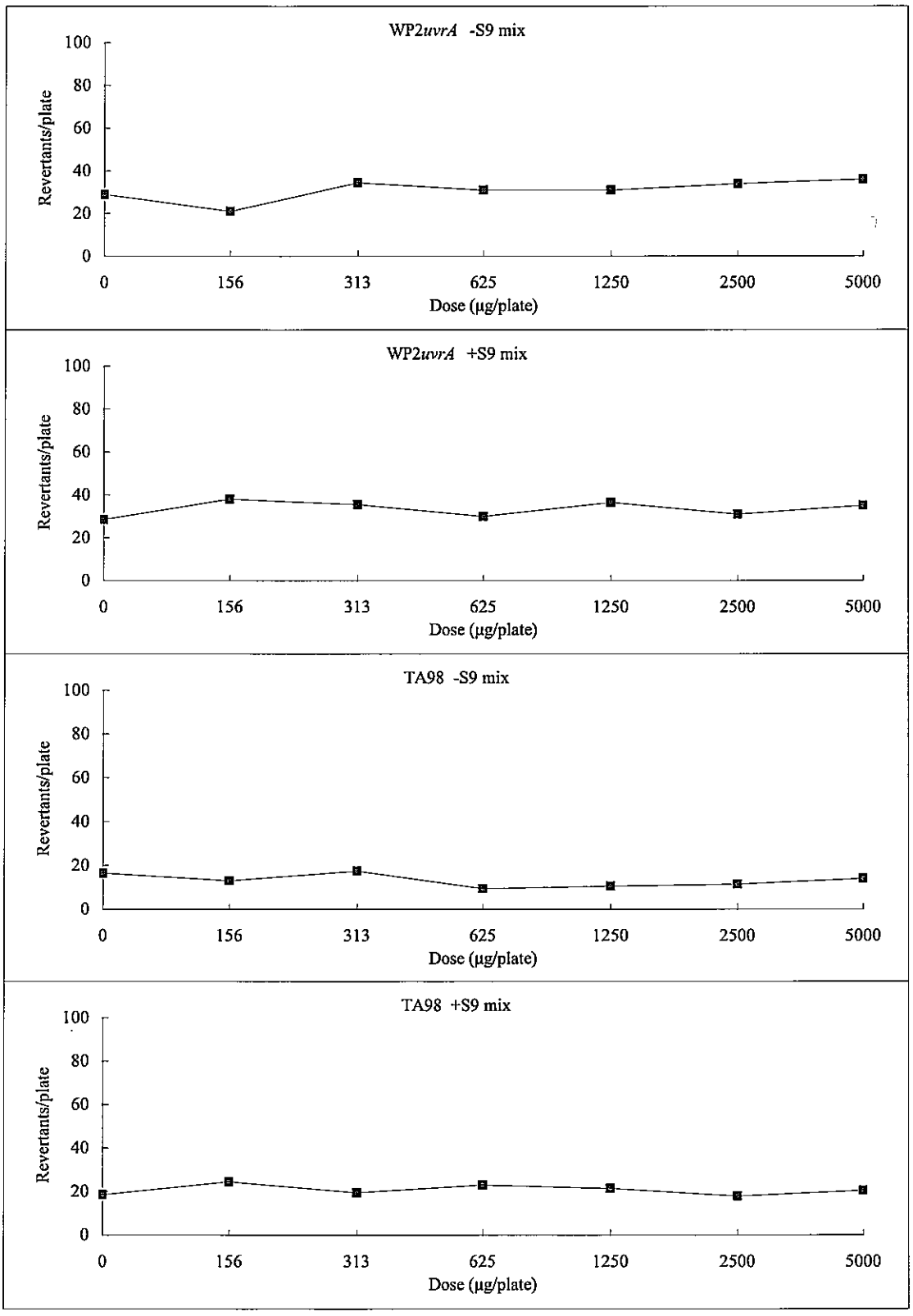
1) AF-2 :2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
2) NaN_3 :Sodiumazide
3) 9-AA :9-Aminoacridine
4) 2-AA :2-Aminoanthracene

[Notes]
1. When toxicity was observed, "*" was placed to the right of the number of the revertants.
2. When precipitation was observed, "†" was placed to the right of the test substance concentration.
3. The average number of revertants in each concentration was showed in ( ).

Dose-response curves (Mutagenicity test)



Dose-response curves (Mutagenicity test)



Dose-response curves (Mutagenicity test)