

AR 226 - 3266

REPORT ON MUTAGENICITY TEST RESULTS USING MICROORGANISMS

1. General Information

Nomenclature of novel chemical substance (by IUPAC nomenclature)	[REDACTED]		
Other name	[REDACTED]		
Structure or empirical formula (outline of preparation method when not certain)	[REDACTED]		
Purity of novel chemical substance used in test	[REDACTED]	Lot No. of novel chemical substance used in test	[REDACTED]
Impurity name and content	[REDACTED]		
CAS No.	34455-29-3	Vapor pressure	-
Molecular weight	570.37	Distribution coefficient	-
Melting point	-	Appearance at room temperature	Brown viscous solid
Boiling point	-		
Stability	Unknown		
Solubility in solvent, etc.	solvent	Solubility	Stability in solvent
	water	<50 mg/mL ^{*2}	-
	DMSO	<50 mg/mL ^{*2}	*4
	acetone	-	-
	Other (*3)	*3	-

[Notes]

Hydrolysis property: unknown

*2 Measured on site at Hita Works

*3 Ethanol soluble (solubility unknown)

*4 50-mg/mL suspension showed no discoloration, heat emission, etc., up to 2 h after preparation, thus judged stable (confirmed on site at Hita Works).

[Note: no pages 2 and 3]

6. Minimum-glucose agar plate medium

Self-made or acquired	1. Self-made ② Acquired (producer: Oriental Kobo Kogyo K.K.)
Production date	November 8, 2002
Lot No. for acquired specimen	ANI740KR
Name of agar used, manufacturer, Lot No.	—

7. Testing method

(1) Testing method and reason for its selection

Testing method selected	① Preincubation method 2. Plate method 3. Other ()
Reasons for selection in the case of "Other"	—

(2) Testing conditions

Composition	Bacterial suspension	0.1 mL
	Test substance solution	0.1 mL
	Na phosphate buffer solution (in direct method)	0.5 mL
	S9 mix (in metabolism activation method)	0.5 mL
	Top agar	2.0 mL
	Other (—)	—
Preincubation	Temperature	37 ± 0.5°C
	Time	20 min
Incubation	Temperature	37 ± 0.5°C
	Time	48 h

8. Colony counting method

Counting method	① Manual ② Instrumental
Correction	① None 2. Yes (correction method: area and number drop)

9. Test results

(1) Test results are shown in a separate table

(2) Evaluation of results

Evaluation (circled)	Positive <u>Negative</u>
Reasons for evaluation Regardless of the presence or absence of S9 mix, against all bacteria, the number of back-mutation colonies was less than 2 times the negative control, thus mutagenicity was judged to be negative.	

(3) Notes

• Dosage setting

Dosage setting experiment-1

With a maximum of 5000 µg/mL, an overall 6-step dosage was set with a nominal dilution ratio of 4.

Dosage setting experiment-2

As a result of dosage setting experiment-1, regardless of the S9 mix, growth inhibition was realized against all test bacteria. More than 4 doses for the effective dosage showing no growth inhibition could not be obtained with TA100, TA1535, and TA1537 in the absence of the S9 mix. For the dosage-setting experiment evaluation and dosage setting of this test with the effective dosage established, with a maximum dosage of 313 µg/plate showing growth inhibition, 6-step dosage was set at a nominal dilution ratio of 4.

Main test

From the dosage-setting experiment results, with the maximum dosage showing growth inhibition, in the absence of the S9 mix, the 313-µg/plate for TA100, TA1535, and TA1537, and the 1250-µg/plate for WP2 uvrA and TA98, the 6-step dosage was set with a nominal dilution ratio of 2. In the presence of the S9 mix, the total [overall] 6-step dosage was set with a nominal dilution ratio of 2 at the most with the 1250-µg/plate for TA100, TA1535, and TA1537 and the 5000-µg/plate for WP2 uvrA and TA98.

- Growth inhibition was observed at a dosage above 156 µg/plate for TA100, above 313 µg/plate for TA1535 and TA1537, and above 1250 µg/plate for WP2 uvrA and TA98; in the absence of S9 mix, and above 1250 µg/plate for TA100, TA1535, and TA1537 and 5000 µg/plate for WP2 uvrA and TA98 in the presence of S9 mix.
- No precipitation of the test substance occurred.
- Number of back-mutation colonies of the negative control and positive control was within the historical data range. No staining in test system occurred, indicating that the testing was a proper procedure.

10. Others

Testing facilities	Name	Hita Works, Kagakubusshitsu Heikakenkyukiko
	Address	3-822 Ishii, Hita, Oita 877-0061 ☎ 0973(24)7211 FAX: 0973(23)9800
Principal tester	Post and name	Testing Department 3 Shozo Ogura
	Experience	21 years
Test No.	K01-2869	
Test duration	2/4/03 to 3/5/03	

Test results (dosage setting experiment-1)

Test substance name [REDACTED]

Test duration		2/4/03 to 2/6/03									
Metabolism activation	Test substance dosage (µg/plate)	Back mutation (No. of colonies/plate)									
		Base pair substitution						Frame shift			
		TA100		TA1535		WP2uvrA		TA98		TA1537	
		111 98	99 (103)	7 13	4 (8)	35 27	23 (28)	15 21	22 (19)	6 5	3 (5)
- S9 mix	Negative control	109 100	(105)	11 16	(14)	19 28	(24)	22 17	(20)	8 4	(6)
	4.88	97 95	(96)	14 11	(13)	25 25	(25)	13 23	(18)	5 2	(4)
	19.5	101 105	(103)	15 11	(13)	25 30	(28)	18 20	(19)	4 6	(5)
	78.1	88* 79*	(84)	3* 3*	(3)	25 26	(26)	17 12	(15)	2* 1*	(2)
	313	81* 74*	(78)	6* 5*	(6)	21* 24*	(23)	22* 18*	(20)	3* 4*	(4)
	1250	59* 75*	(67)	2* 0*	(1)	29* 18*	(24)	15* 16*	(16)	3* 2*	(3)
	5000	104 120	90 (105)	8 10	10 (9)	32 37	30 (33)	40 31	25 (32)	16 14	17 (16)
	4.88	89 97	(93)	5 16	(11)	26 21	(24)	33 27	(30)	18 19	(19)
+ S9 mix	Negative control	97 112	(105)	13 14	(14)	38 28	(33)	23 32	(28)	15 21	(18)
	4.88	99 107	(103)	17 9	(13)	31 25	(28)	25 24	(25)	17 18	(18)
	19.5	107 108	(108)	11 8	(10)	30 30	(30)	35 29	(32)	13 19	(16)
	78.1	101* 106*	(104)	16* 12*	(14)	25 27	(26)	31 34	(33)	10* 8*	(9)
	313	83* 68*	(76)	2* 5*	(4)	28* 25*	(27)	15* 16*	(16)	3* 4*	(4)
	1250	AF-2		NaN ₃		AF-2		AF-2		ICR-191	
	5000	0.01		0.5		0.01		0.1		0.5	
	Colony/plate	257 261	(259)	278 304	(291)	184 174	(179)	510 532	(521)	1000 1054	(1027)
Positive control	Without S9 mix	Name		2AA		2AA		2AA		2AA	
	With S9 mix	Dosage (µg/plate)		1		2		10		0.5	
		Colony/plate		395 307		142 120		742 797		476 482	
				(351)		(131)		(770)		(479)	

[Notes]

(): average value of number of colonies of each plate

*: growth inhibition of bacteria was observed.

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

NaN₃: sodium azide

ICR-191: 2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]acridine•2HCl

2AA: 2-aminoanthracene

Test results (dosage setting experiment-2)

Test substance name [REDACTED]

Test duration		2/7/03 to 2/10/03						
Metabolism activation		Test substance dosage (µg/plate)	Back mutation (No. of colonies/plate)					
			Base pair substitution				Frame shift	
			TA100		TA1535		TA1537	
- S9 mix	Negative control	96 95	102 (98)	12 6	6 (8)	6 5	12 (8)	
	0.305	110 106	(108)	13 10	(12)	6 4	(5)	
	1.22	89 96	(93)	9 10	(10)	5 8	(7)	
	4.88	95 86	(91)	5 12	(9)	8 2	(5)	
	19.5	113 100	(107)	8 6	(7)	7 7	(7)	
	78.1	106 99	(103)	7 12	(10)	7 9	(8)	
	313	95* 82*	(89)	5* 6*	(6)	4* 5*	(5)	
	Positive control	Without S9 mix	Name	AF-2		NaN ₃		ICR-191
Dosage (µg/plate)			0. 01		0. 5		0. 5	
Colonies/plate			244 228 (236)		351 354 (353)		893 852 (873)	

[Notes]

(): average value of number of colonies of each plate

*: growth inhibition of bacteria was observed.

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

NaN₃: sodium azide

ICR-191: 2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]acridine•2HCl

Test results (main test)

Test substance name: [REDACTED]

Test duration		2/13/03 to 2/18/03									
Metabolism activation	Test substance dosage (µg/plate)	Back mutation (No. of colonies/plate)									
		Base pair substitution					Frame shift				
		TA100		TA1535		WP2uvrA	TA98		TA1537		
		107	115	11	9	28	29	21	27	7	5
- S9 mix	Negative control	107	(110)	11	(10)	28	(28)	21	(22)	7	(5)
	9.77	111		13		—		—		7	
	19.5	103	(107)	8	(11)	—		—		3	(5)
	39.1	114		7		—		—		8	
	78.1	119	(117)	8	(8)	—		—		6	(7)
	156	110		7		27		16		10	
	313	127	(119)	6	(7)	23	(25)	19	(18)	8	(9)
	625	110		9		30		18		7	
	1250	98	(104)	8	(9)	26	(28)	19	(19)	9	(8)
		107*		7		23		16		5	
+ S9 mix	Negative control	73*	(90)	4	(6)	24	(24)	23	(20)	6	(6)
	39.1	102*		6*		24		10		6*	
	78.1	104*	(103)	3*	(5)	23	(24)	17	(14)	2*	(4)
	156	—		—		18		23		—	
	313	—		—		21	(20)	21	(22)	—	
	625	—		—		30*		13*		—	
	1250	—		—		22*	(26)	14*	(14)	—	
		119	114	9	9	29	29	41	45	13	19
	39.1	118	(117)	11	(10)	33	(30)	46	(44)	15	(16)
	78.1	106		12		—		—		17	
Positive control	Without S9 mix	126	(116)	6	(9)	—		—		18	(18)
	With S9 mix	124		11		—		—		26	
		117	(121)	3	(7)	—		—		19	(23)
		123		12		32		36		16	
		133	(128)	6	(9)	29	(31)	39	(38)	14	(15)
		104		9		35		35		22	
		129	(117)	9	(9)	27	(31)	27	(31)	24	(23)
		90		15		36		43		25	
		108	(99)	9	(12)	33	(35)	35	(39)	16	(21)
		95*		8*		33		32		19*	
Positive control	Without S9 mix	100*	(98)	7*	(8)	27	(30)	39	(36)	21*	(20)
	With S9 mix	—		—		35		31		—	
		—		—		40	(38)	42	(37)	—	
		—		—		34*		5*		—	
		—		—		33*	(34)	15*	(10)	—	
		Name	AF-2	NaN ₃		AF-2		AF-2		ICR-191	
		Dosage (µg/plate)	0.01	0.5		0.01		0.1		0.5	
		Colonies/plate	300	275		162		613		825	
			287	280	(278)	200	(181)	572	(593)	696	(761)
		Name	2AA	2AA		2AA		2AA		2AA	
Positive control	Without S9 mix	Dosage (µg/plate)	1	2		10		0.5		2	
	With S9 mix	Colonies/plate	861	175		534		481		263	
			682	177	(176)	509	(522)	493	(487)	293	(278)

[Notes]

(): average value of number of colonies of each plate

*: growth inhibition of bacteria was observed.

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

NaN₃: sodium azide

ICR-191: 2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]acridine•2HCl

2AA: 2-aminoanthracene

Dosage-Reaction Curve (dosage setting experiment-1)

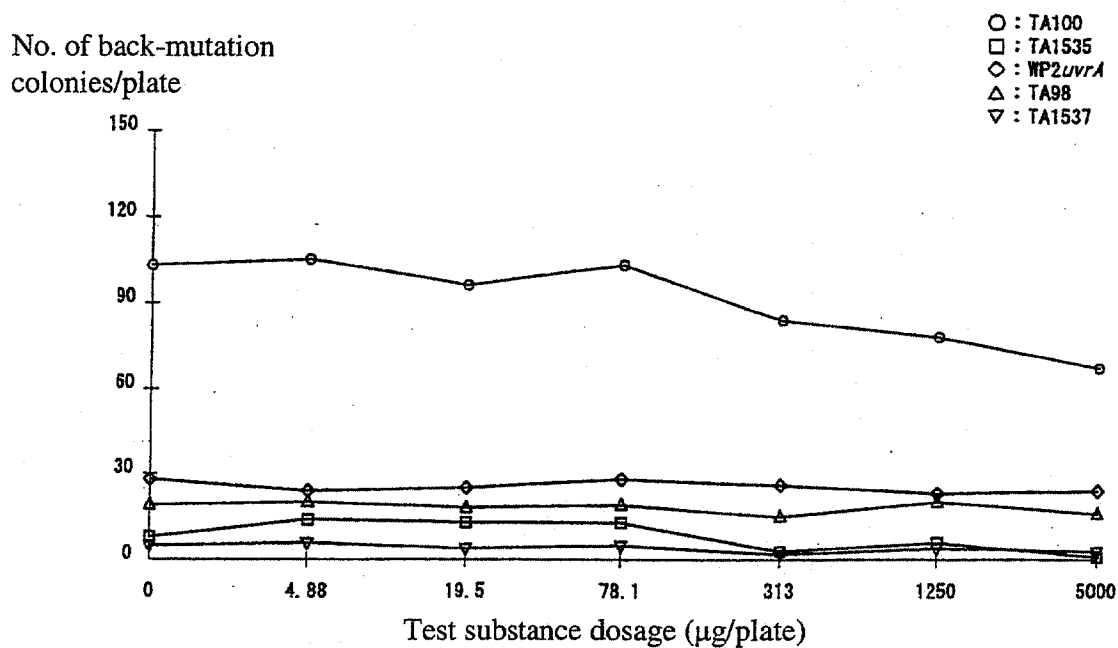


Figure 1: Dosage-reaction curve without S9 mix

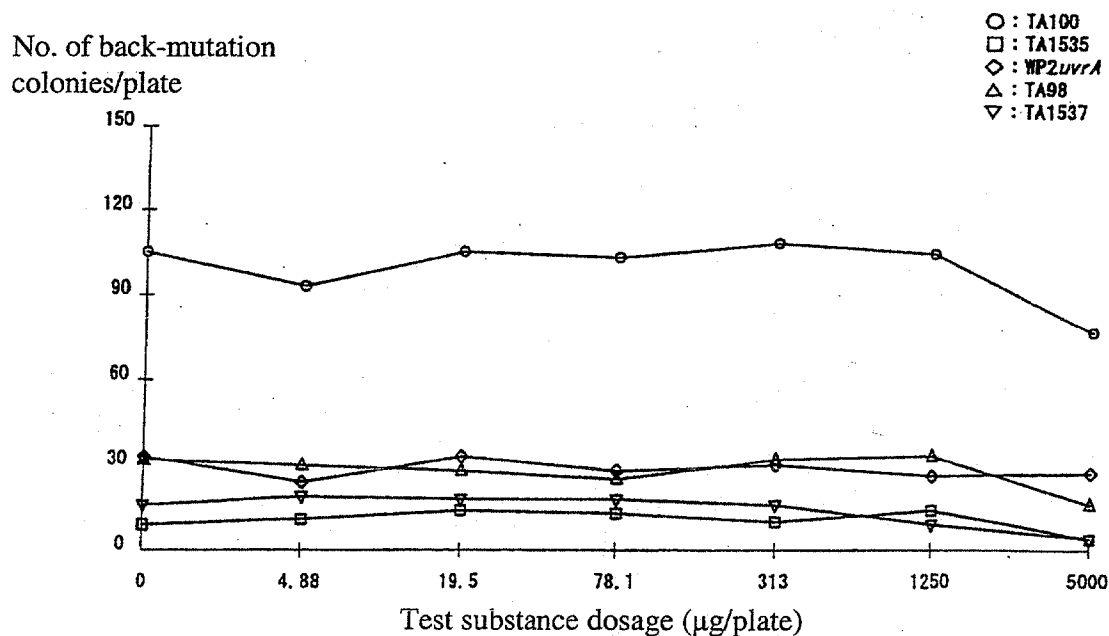


Figure 2: Dosage-reaction curve with S9 mix

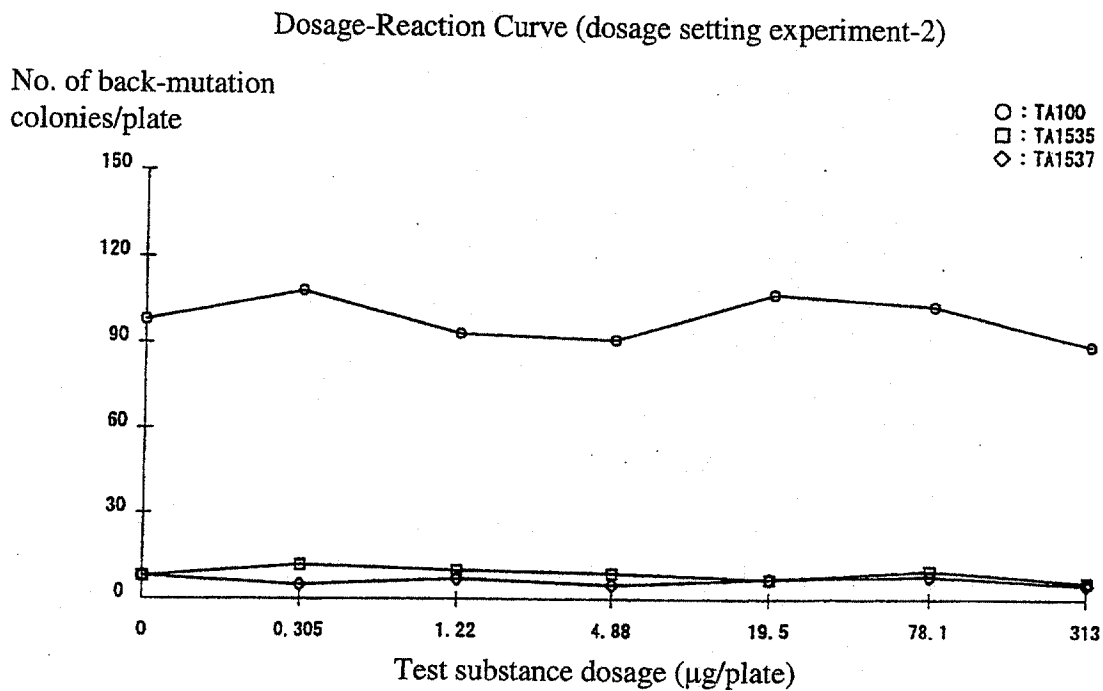


Figure 3: Dosage-reaction curve without S9 mix

Dosage-Reaction Curve (main test)

No. of back-mutation colonies/plate

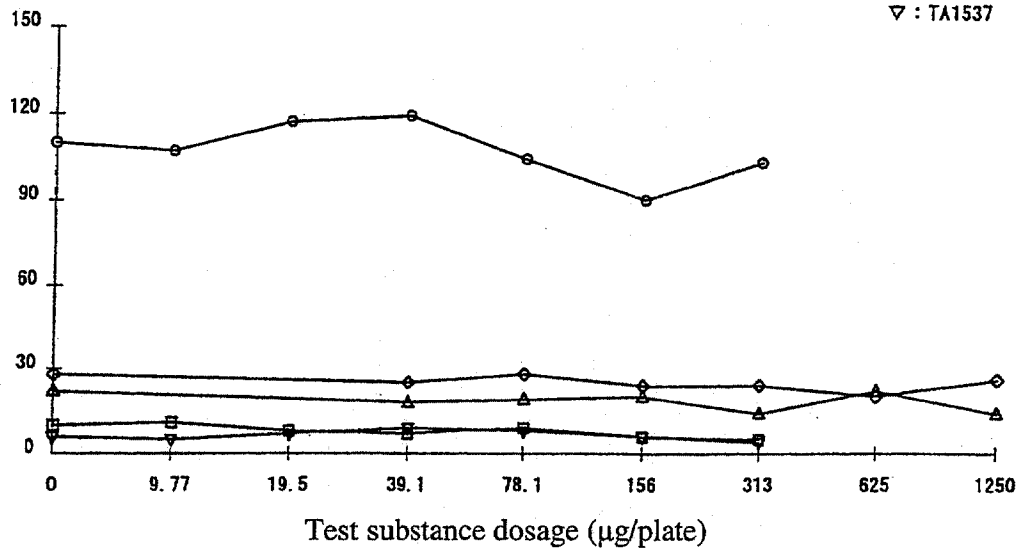


Figure 4: Dosage-reaction curve without S9 mix

No. of back-mutation colonies/plate

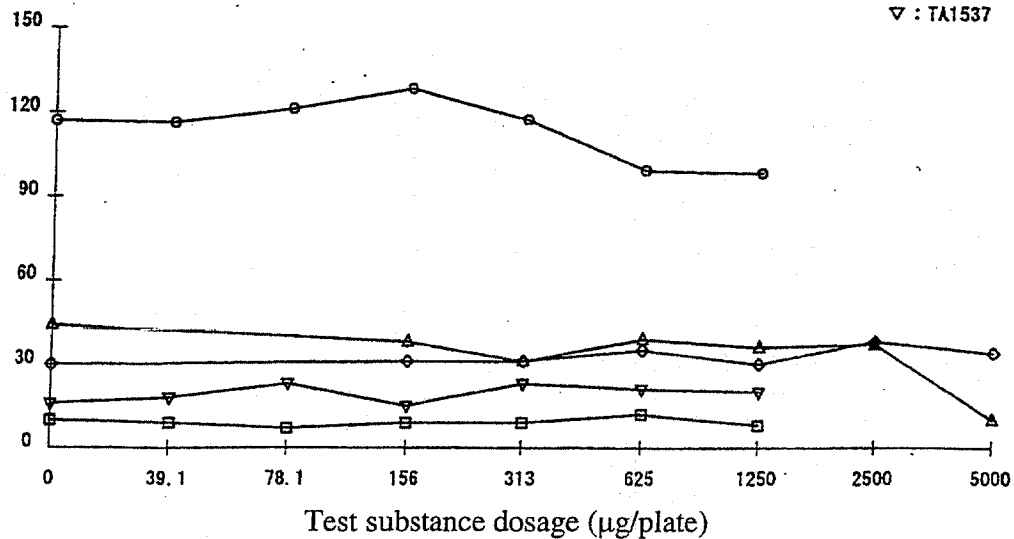


Figure 5: Dosage-reaction curve with S9 mix

Kiatsu No. 90
February 22, 2001

Jiro Hiraishi
Chairman, Board of Directors
Kagakubusshitsu Heikakenkyukiko

Labor Standards Bureau
Ministry of Health, Labor and Welfare

Safety-Health Law GLP Feasibility Confirmation of Test Facilities

This is notification of the following judgments of the title matter requested on March 24, 2000.

Description

- 1 Name of test facilities for feasibility confirmation
Hita Works, Kagakubusshitsu Heikakenkyukiko
- 2 Title of test requiring feasibility confirmation
Mutagenicity test using microorganisms and chromosomal aberration test using mammalian cultured cells
- 3 Inspection date
July 24, 2000 to July 25, 2000
- 4 Judgment
Pass
- 5 Effective duration of feasibility confirmation
July 26, 2000 to July 25, 2003
- 6 Notes
None

STATEMENT

Hita Works
Kagakubusshitsu Heikakenkyukiko

Test Requester: Dupont K.K.
Test Title: Mutagenicity test of [REDACTED] using microorganisms
Test Code No.: K01-2869

The test described above was carried out in accordance with Standards for Facilities for Toxicity Test (1988 Ministry of Labor Bulletin No. 76, partially amended on March 29, 2000), Test Facilities Specified in Ministry Order Article 4: Defining Tests for New Chemical Substances and Toxicity Test of Designated Chemical Substances (March 31, 1984, Kanhogyo No. 39, Yakuhatu No. 229, 59 Kikyoku No. 85, amended on March 1, 2000), and OECD Principles of Good Laboratory Practice (November 26, 1997).

Yoshifumi Fujino
Manager
March 5, 2003

STATEMENT

Hita Works
Kagakubusshitsu Heikakenkyukiko

Test Requester: Dupont K.K.
Test Title: Mutagenicity test of [REDACTED] using microorganisms
Test Code No.: K01-2869

The test described above was carried out in accordance with Standards for Facilities for Toxicity Test (1988 Ministry of Labor Bulletin No. 76, partially amended on March 29, 2000), Test Facilities Specified in Ministry Order Article 4: Defining Tests for New Chemical Substances and Toxicity Test of Designated Chemical Substances (March 31, 1984, Kanhogyo No. 39, Yakuhatu No. 229, 59 Kikyoku No. 85, amended on March 1, 2000), and OECD Principles of Good Laboratory Practice (November 26, 1997).

The final report precisely reflects raw data, and it is to confirm the test data as being effective.

Shozo Ogira
Principal Tester
March 5, 2003

RELIABILITY WARRANTY

Hita Works
Kagakubusshitsu Heikakenkyukiko

Test Requester: Dupont K.K.

Test Title: Mutagenicity test of [REDACTED] using microorganisms

Test Code No.: K01-2869

This test has been audited or inspected by the QA Division of Hita Works, Kagakubusshitsu Heikakenkyukiko. Supervision or inspection dates and reporting dates to the principal tester and management are given below.

Auditing or inspection items	Auditing or inspection date	Auditing or inspection result report date
Test plan documented	2/5/2003	2/6/2003
Test substance preparation and test start	2/13/2003	2/14/2003
Amendment of test plan document	2/17/2003	2/17/2003
Amendment of test plan document (No. 2)	2/19/2003	2/19/2003
Recording and report draft	2/25/2003	2/25/2003
Final report draft review	3/5/2003	3/5/2003
Final report	3/5/2003	3/5/2003

The test described above was carried out in accordance with Standards for Facilities for Toxicity Test (September 1, 1988 Ministry of Labor Bulletin No. 76, partially amended on March 29, 2000), Test Facilities Specified in Ministry Order Article 4: Defining Tests for New Chemical Substances and Toxicity Test of Designated Chemical Substances (March 31, 1984, Kanhogyo No. 39, Yakuhatu No. 229, 59 Kikyoku No. 85, amended on March 1, 2000), and OECD Principles of Good Laboratory Practice (November 26, 1997) and in accordance with test methods defined in Toxicity Test Standards (Ministry of Labor Bulletin No. 77, September 1, 1988; Ministry of Labor Bulletin No. 67, June 2, 1997), Specific Procedure of Mutagenicity Test Using Microorganisms (February 8, 1999), Testing Methods for New Chemical Substances,

partial amendment (Kanhogyo No. 700, Yakuhatu No. 1039, 61 Kikyoku No. 1014, December 5, 1986; Kanhoan No. 287, Eisei No. 127, 1997-10-31 Kikyoku No. 2, October 31, 1997).

This report accurately describes the testing method and procedure; the reported results accurately reflect raw data of the tests.

March 5, 2003

Keiji Shiraishi

Reliability Warranty Officer

Yoshifumi Fujino

Manager

March 5, 2003

Language Services Unit

Phoenix Translations

January 13, 2004