Mutagenicity Testing of

2-[N-ethyl-N-perfruoloalkyl(C=1~8)sulfonylamino]ethylacrylate

in Bacterial Reverse Mutation Assays

(study number 2862)

T-6322.5

Sample P-1

FX-13

Mutagenicity

test

B M L, INC.

QUALITY ASSURANCE CERTIFICATE

Title of Study : Mutagenicity testing of 2-[N-ethyl-N-perfruoloalkyl(C=1 ~8) sulfonylamino] ethylacrylate in bacterial reverse mutation assays

Study Number : 2 8 6 2

This study was conducted in compliance with " Standards to be Observed by Mutagenicity Testing Institutions "(Notification No. 76, Ministry of Labour, Japan, September 1, 1988) and " GLP Standards applied to Industrial Chemicals " (Notification No. 39 of the Planning and Coordination Bureau, Environment Agency, No. 229 of the Pharmaceutical Affairs Bureau, Ministry of Health and welfare & No.85 of the Basic Industries Bureau, Ministry of international Trade and Industry, Japan, 18, 1988).
I certify that the methods and procedures used in the test are described precisely in the final report, and that the reported results reflect the raw data accurately.
The circumstances of audits and inspections areas follows.

Date of Q. A.	*Item*	*Date of Report Inspection to Management*
Mar. 23, 1991	*Review of SOP and protocol*	*Apr. 4, 1991*
Apr. 10, 1991	*General inspection*	*Apr. 12, 1991*
Apr. 18, 1991	*Review of draft report and audit of raw data*	*Apr. 18, 1991*
Apr. 24, 1991	*Review of final report (written in Japanese)*	*Apr. 24, 1991*
Apr. 8, 1996	*Review of final report (written in English)*	*Apr. 8, 1996*

Quality Assurance Unit
Safety Evaluation Department
BML, Inc.

M. Kuramochi

Masahiro Kuramochi
Title: Manager

Date: Apr. 8, 1996

QUALITY ASSURANCE STATEMENT

Title of Study : Mutagenicity testing of 2-[N-ethyl-N-perfruoloalkyl(C=1 ~8) sulfonylamino] ethylacrylate in bacterial reverse mutation assays

Study Number : 2 8 6 2

This report has been audited by Quality Assurance Personnel and is considered to represent a true and accurate translation of the original report No. 2862, written in Japanese, issued on April 24, 1996, partially amended since March 8, 1995,by BML, Inc..

Date : April 8, 1996

Position : ***Manager, Quality Assurance Unit, Safety Evaluation Department BML, Inc.***

(Company Name and Title)

Name : ***Masahiro Kuramochi*** M. Kuramochi

(Type) ***(Signature)***

STATEMENT OF STUDY DIRECTOR

Title of Study : A mutagenicity testing of 2-[N-ethyl-N-perfruoloalkyl (C=1～8)sulfonylamino]ethylacrylate in bacterial reverse mutation assays

Study Number : 2862

This is to certify that the above study was conducted in compliance with "Standards to be Observed by Mutagenicity Testing Institutions" (Notification No.76, Ministry of Labour, Japan, September 1, 1988) and "GLP Standards Applied to Industrial Chemicals" (Notification No.39 of the Planning and Coordination Bureau, Environment Agency, No.229 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare & No.85 of the Basic Industries Bureau, Ministry of International Trade and Industry, Japan, November 18, 1988).

Date : April 8, 1996

Position : Study Director, Mutagenesis Tests Division
BMl, INC.

(Company Name and Title)

Name : Yoshihiro Oguma

(Type)

Yoshihiro Oguma

(Signature)

Study number : 2862

Title of Study : A mutagenicity testing of 2-[N-ethyl-N-perfruoloalkyl (C=1～8)sulfonylamino]ethylacrylate in bacterial reverse mutation assays

Purpose of the test:

To assess the effect of the test substance in bacterial reverse mutation assays.

This study was performed in accordance with the guidelines set forth in "Standards for Mutagenicity Tests using Microorganisms"(Notification No.77, Ministry of Labour, Japan, September 1, 1988) and "Guidelines for Toxicity Testings of Chemicals" (Notification No. 237 of the Planning and Coordination Bureau, Environment Agency, No. 306 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare & 303 of the Basic Industries Bureau, Ministry of International Trade and Industry, Japan, March 31, 1987).

And it was conducted in compliance with "Standards to be Observed by Mutagenicity Testing Institutions" (Notification No.76, Ministry of Labour, Japan, September 1, 1988) and "GLP Standards Applied to Industrial Chemicals" (Notification No.39 of the Planning and Coordination Bureau, Environment Agency, No.229 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare & No.85 of the Basic Industries Bureau, Ministry of International Trade and Industry, Japan, November 18, 1988).

Sponcer

Name : Sumitomo 3M Limited
Address : 3-8-8 Minamihashimoto, Sagamihara-shi, Kanagawa 229, Japan

Testing facility

Name : BML,INC.
Address : 1361-1 Matoba, Kawagoe-shi, Saitama 350-11, Japan

Date of test

Initiation : March 18, 1991
Completion : April 24, 1991

Persons in charge

Study Director : Yoshihiro Oguma
Persons in charge of study : Shigemi Kimura, Youko Hasegawa

Name of the person who prepared the final report, and date of preparation

Yoshihiro Oguma

Title: Study Director
Date : April 24, 1991

I certify that this study was conducted under the specified protocol.

Munehiro Terasoma

Title: Administrator
Date : April 24, 1991

1. Summary

Reverse mutation assays using *Salmonella typhimurium* strains TA100, TA1535, TA98 and TA1537 and *Escherichia coli* WP2 *uvr*A were conducted on 2-[N-ethyl-N-perfruoloalkyl(C=1～8)sulfonylamino]ethylacrylate with and without metabolic activation.

As a result, the test substance did not induce significant revertants to be compared with the solvent controls and dose-dependent increase was not found in any strains with or without metabolic activation.

It was concluded that 2-[N-ethyl-N-perfruoloalkyl(C=1～8)sulfonylamino] ethylacrylate was not mutagenic to bacteria under the experimental conditions.

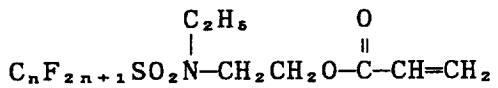
2. Date of study

Initiation of the dose-finding test : March 28, 1991
Initiation of the main test : April 9, 1991
Completion of the main test : April 12, 1991

3. Test substance

(1) Test substance

1) Name{Another name} : 2-[N-ethyl-N-perfruoloalkyl(C=1～8)sulfonyl amino]ethylacrylate {Sample D-1}
2) BML-code : BML-1107
3) Lot No. : 103
4) Purity : greater than 99 % wt/wt
5) Name and concentration of impurities
: phenothyadine 60± 20ppm
hydroquinonemonomethylether 170± 35ppm
6) Structural formula :



n=1～ 8
n= 8 (main component) : ca. 78 %
n= 1 ～ 7 : ca. 21 % (total)

7) Molecular weight : 625
8) Appearance at room temperature
: amber wax
9) Melting point : 27-42℃
10) Boiling point : ca.150℃ (1mmHg)
11) Vapor pressure : information not provided
12) Solubility : Oil soluble
13) Degree of solubility
Water : insoluble
Dimethylsulfoxide (DMSO) : insoluble
Acetone : over 50 %
14) Partition coefficient
: information not provided
15) Stability : stable

The test substance was analysed and all data are retained by the client.

(2) Solvent

Name	: Acetone
Manufacturer	: Wako Pure Chemical Industries, Ltd.
Lot No.	: ECG2489
Purity	: greater than 99% (Special grade of JIS standard)

4. Controls

(1) Negative control

Negative controls were treated with Acetone only.

(2) Positive control compounds

The names and concentrations of these compounds are given in the table below.

Strain	without S9(μg/plate)		with S9(μg/plate)	
TA100	AF-2	(0.01)	B(a)P	(5.0)
TA1535	NaN_3	(0.5)	2AA	(2.0)
TA98	AF-2	(0.1)	B(a)P	(5.0)
TA1537	ICR-191	(1.0)	B(a)P	(5.0)
WP2 *uvrA*	AF-2	(0.01)	2AA	(10.0)

AF-2 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
(Wako Pure Chemical; Lot No. LAJ1702; Special grade of JIS standard, Purity greater than 95 %)

NaN_3 Sodium azide
(Wako Pure Chemical; Lot No. DCJ6398; Special grade of JIS standard, Purity greater than 95 %)

ICR-191 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino] acridine ·2HCl
(Polysciences,Inc.; Lot No. 52300; Purity greater than 95 %)

2AA 2-Aminoanthracene
(Wako Pure Chemical; Lot NO. KPQ0892; Special grade of JIS standard, Purity greater than 95 %)

B(a)P Benzo(a)pyrene
(Wako Pure Chemical; Lot No. AWK3751; Special grade of JIS standard, Purity greater than 95 %)

Sodium azide(NaN_3) was dissolved in distilled water(Hikari Pharmacy Co., Ltd.;Lot No. 9003VA), the other compounds were dissolved in DMSO (Wako Pure Chemical Industries Ltd.;Lot No. AWE6768, ECM4254). Compounds mutagenic to each strain were used for positive controls; these were chosen according to the "New Guide Book of Mutagenicity Test Using Microorganisms," the Ministry of Labour, Japan (1986).

5. Materials

(1) Tester strains

1) Strains used [1),2),3)]

Base-pair substitution type :

S.typhimurium TA100, TA1535

E.coli WP2 *uvr*A

Frameshift type :

S.typhimurium TA98, TA1537

2) Supplier and date obtained

S.typhimurium TA100

: National Institute of Hygienic Sciences, Japan; April 15, 1982

S.typhimurium, other TA strains

: Dr. Ames, U.C. Berkeley, CA, U.S.A.; January 20, 1988

E.coli WP2 *uvr*A

: Department of Molecular Oncology, Institute of Medical Science, University of Tokyo, Japan; November 24, 1987

3) Reason for choice

These strains are well established as sensitive to many classes of mutagens and are widely used.

4) Condition of storage

The cell suspension was mixed with dimethylsulfoxide(spectrophotometric grade in a ratio of 0.07 to 0.8 volume and stored at -80℃.

(2) S9 Mix

1) S9

Name:	S9
Supplier:	Kikkoman Co.
Lot No.:	RAA-248
Prepared on:	January 11, 1991
Purchased on:	January 25, 1991
Species, Strain:	Sprague-Dawley Rat
Sex:	Male
Age:	7 weeks
Weight:	199-246g
Inducing substance:	Phenobarbital(PB) and 5,6-benzoflavone(BF), i.p. injection
Dose:	PB=30+60+60+60 mg/kg (4 days); BF=80 mg/kg(1 day)

2) Cofactor

Name:	Cofactor
Supplier:	Boehringer Mannheim Yamanouchi K.K.
Lot No.:	711

3) Composition of S9 Mix (per 1ml)

S9 fraction	0.1 ml
$MgCl_2$	8.0 μmol
KCl	33.0 μmol
G-6-P	5.0 μmol
NADPH	4.0 μmol
NADH	4.0 μmol
Na-Phosphate buffer (pH 7.4)	100.0 μmol

(3) Minimum glucose agar plates

Name	: Minimal glucose agar plates M
Supplier	: Nisshin Flour Milling Co., Ltd.
Lot No.	: BM020CG
Prepared on	: March 1, 1991
Purchased on	: March 11, 1991
Storage	: stored at room temperature

6. Methods

(1) Preparation of the test substance

By reason of information from sponsor, the test substance was dissolved and tested in Acetone. The concentration of the substance was doubled(100mg/ml) and half(0.05ml) of this solution was added to reduced the toxicity of Acetone. Its solution was stored in refrigerator just prior to use.

(2) Dose selection

The dose range was determined from the dose-finding tests using the following seven dose levels: 1.2, 4.9, 20, 78, 313, 1250 and 5000 μg/plate.

In the dose-finding test, growth inhibition was not found in all strains with or without metabolic activation.

Accordingly, we selected 5000 μg/plate as the maximum dose for all strains tested with or without metabolic activation. So for all tester strains, we used five doses serially 1 : 2 were generally used.

(3) Test procedure (preincubation method) [4),5),6)]

The cells of each strain were inoculated into a culture tube containing 10 ml of Nutrient Broth No.2 (Oxoid) and these were cultured to the early stationary phase of growth, shaking(100rpm) for 8 h at 37℃.

For tests without metabolic activation, 0.5ml of 0.1M Na-phosphate buffer(pH7.4) was added to each tube containing 0.05 ml of the test substance, and 0.1 ml of each fresh bacterial medium was added to each test tube. For tests with metabolic activation, 0.5ml of S9 mix instead of 0.5ml of 0.1M Na-phosphate buffer(pH7.4) was added to each tube containing 0.05 ml of the test substance. The mixture was preincubated for 20 min in a water bath at 37℃. 2 ml of top agar was then added to the mixture, and the contents of each tube were mixed and poured over the surface of the minimum glucose agar plate. The overlay agar was allowed to solidify at room temperature.

All plates were incubated for 48 h at 37℃ and then the number of revertant colonies were counted. Afterward, bacterial growth inhibition was checked using a stereoscopic microscope.

Added 1:10 to the top agar (0.6% Bacto-agar, 0.5% NaCl) that were melted by warming just prior to use, were 0.5mM L-histidine and 0.5mM biotin for the *S. typhimurium* strains, and 0.5mM tryptophan solution for the *E. coli* strain. All plating were done in duplicate.

7. Criteria of evaluation [5)]

If a significant, reproducible increase in the number of revertants (twice that of the solvent control) was detected in at least one of the test points, or a reproducible dose-related increase occurred over more than one point, the results would be considered positive. Statistical method was not used as an aid in evaluating the test results.

8. Results and discussion

The test results are shown in Tables 1, 2 and Figs.1, 2. The figures were prepared from Table 2.

In the dose-finding tests and the main tests, the test substance did not induce revertants more than twice that of the solvent controls and dose-dependent increase was not found in any strains with or without metabolic activation.

The positive controls showed an increase in the number of revertants compared to the solvent controls, indicating that this study was performed properly.

From these results, it was concluded that this test substance was negative.

No growth inhibition was found in any strain. Precipitate on the plates was found at over 313 μg/plate without metabolic activation and over 1.2 μg/plate with metabolic activation.

The sterile tests performed on both the test substance and the S9 mix indicated no bacterial contamination throughout this experiment.

9. Deviation from protocol,etc.

During the study period, there were no environmental factors considered to have affected the reliability of the data, and there was no deviation from the protocol.

10. Conclusion

From the above results, it was concluded that 2-[N-ethyl-N-perfruoloalkyl (C=1～8)sulfonylamino]ethylacrylate was non-mutagenic in bacterial mutation assays under the experimental conditions.

11. Storage of the records and samples

All records and samples generated during this study (listed below) will be stored in the archival area of BML,INC. for 10 years after the tests. At the end of the 10-year period, the sponsor will be consulted regarding the disposal or continued storage of the records and samples.

1) Protocol and Amendments
2) Records concerning the test substances
3) Raw data
4) Records on the Activities of Quality Assurance Unit
5) Test substance
6) Draft of the final report
7) The final report

12. References

(1) B.N. Ames, F.D. Lee, and W.E. Durston: An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens, Proc. Natl. Acad.Sci., USA, 70, No.3, pp.782-786, March 1973

(2) J. McCann, N.E. Spingarn, J. Kobori, and B.N. Ames: Detection of Carcinogens as Mutagens: Bacterial Tester Strains with R Factor Plasmids, Proc. Natl Acad. Sci., USA, 72, No.3, pp.979-983, 1975

(3) M.H.L. Green and W.J. Muriel: Mutagen Testing using Trp$^+$ Reversion in *Escherichia coli*, Mutation Res., 38, pp.3-32, 1976

(4) D.M. Maron and B.N. Ames: Revised Methods for the Salmonella Mutagenicity Test, Mutation Res., 113, pp.173-215, 1983

(5) Japanese Ministry of Labour, *in* New Guidebook of Mutagenicity Tests Using Microorganisms, Japan Industrial Safety and Health Association, 1986

(6) Y. Tajima, T. Yoshida, and T. Kada, *in* Screening for Detection of Chemical Mutagens, Koudansha Co., 1973

Table 1

Table of Dose Finding Test Results

Name of test substance : 2-[N-ethyl-N-perfruoloalkyl(C=1~8)sulfonylamino]ethylacrylate

No. 2862

With(+)or without(-) S9Mix	Test substance concentration (μg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
S9Mix (-)	Solvent control	153 174 (164)	14 21 (18)	25 16 (21)	31 24 (28)	10 12 (11)
	1.2	160 177 (169)	18 19 (19)	23 27 (25)	17 22 (20)	14 14 (14)
	4.9	160 175 (168)	25 20 (23)	25 16 (21)	21 18 (20)	11 10 (11)
	20	165 158 (162)	22 24 (23)	27 29 (28)	20 24 (22)	8 9 (9)
	78	149 150 (150)	19 20 (20)	27 33 (30)	26 24 (25)	8 13 (11)
	313	141 # 159 # (150)	14 # 11 # (13)	22 # 23 # (23)	26 # 23 # (25)	12 # 11 # (12)
	1250	141 # 157 # (149)	21 # 14 # (18)	21 # 28 # (25)	17 # 14 # (16)	7 # 7 # (7)
	5000	145 # 180 # (163)	18 # 15 # (17)	29 # 20 # (25)	22 # 24 # (23)	10 # 11 # (11)
S9Mix (+)	Solvent control	145 132 (139)	19 21 (20)	30 31 (31)	28 27 (28)	16 18 (17)
	1.2	120 # 153 # (137)	22 # 10 # (16)	29 # 30 # (30)	22 # 26 # (24)	21 # 21 # (21)
	4.9	180 # 135 # (158)	26 # 13 # (20)	28 # 20 # (24)	19 # 25 # (22)	21 # 18 # (20)
	20	112 # 152 # (132)	20 # 18 # (19)	23 # 24 # (24)	35 # 28 # (32)	20 # 21 # (21)
	78	160 # 145 # (153)	20 # 14 # (17)	25 # 31 # (28)	31 # 31 # (31)	20 # 19 # (20)
	313	147 # 152 # (150)	13 # 22 # (18)	24 # 28 # (26)	31 # 28 # (30)	14 # 22 # (18)
	1250	129 # 117 # (123)	16 # 14 # (15)	30 # 28 # (29)	24 # 32 # (28)	16 # 21 # (19)
	5000	142 # 138 # (140)	22 # 20 # (21)	25 # 31 # (28)	25 # 25 # (25)	17 # 15 # (16)
Positive control not requiring S9Mix	Name	AF-2 #1	NaN_3 #2	AF-2	AF-2	ICR-191 #3
	Concentration (μg/plate)	0.01	0.5	0.01	0.1	1.0
	Number of colonies/plate	723 694 (709)	294 234 (264)	135 123 (129)	570 568 (569)	1019 1099 (1059)
Positive control requiring S9Mix	Name	B[a]P #4	2AA #5	2AA	B[a]P	B[a]P
	Concentration (μg/plate)	5.0	2.0	10.0	5.0	5.0
	Number of colonies/plate	896 789 (843)	185 176 (181)	355 378 (367)	212 174 (193)	99 102 (101)

#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:Benzo[a]pyrene #5:2-Aminoanthracene

#:The test substance was found precipitating on the plates.

Notes:The average number of colonies in each concentration is shown in the ( ).

Table 2

Table of Test Results

Name of test substance : 2-[N-ethyl-N-perfruoloalkyl(C=1～8)sulfonylamino]ethylacrylate

No. 2 8 6 2

With(+)or without(-) S9Mix	Test substance concentration (μg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
S9Mix (−)	Solvent control	140 132 (136)	19 18 (19)	23 35 (29)	22 31 (27)	3 12 (8)
	313	152 # 130 # (141)	16 # 29 # (23)	26 # 22 # (24)	26 # 34 # (30)	7 # 8 # (8)
	625	138 # 147 # (143)	22 # 17 # (20)	18 # 31 # (25)	32 # 24 # (28)	7 # 6 # (7)
	1250	143 # 137 # (140)	14 # 20 # (17)	28 # 26 # (27)	28 # 23 # (26)	14 # 7 # (11)
	2500	136 # 137 # (137)	20 # 25 # (23)	28 # 31 # (30)	30 # 26 # (28)	5 # 7 # (6)
	5000	112 # 95 # (104)	22 # 20 # (21)	21 # 36 # (29)	28 # 36 # (32)	6 # 7 # (7)
S9Mix (+)	Solvent control	145 145 (146)	20 18 (19)	27 25 (26)	38 41 (40)	17 21 (19)
	313	133 # 119 # (126)	14 # 16 # (15)	37 # 27 # (32)	40 # 39 # (40)	19 # 22 # (21)
	625	135 # 146 # (141)	11 # 27 # (19)	28 # 35 # (32)	32 # 26 # (29)	18 # 15 # (17)
	1250	151 # 144 # (148)	15 # 13 # (14)	28 # 25 # (27)	41 # 44 # (43)	19 # 15 # (17)
	2500	123 # 141 # (132)	17 # 17 # (17)	23 # 39 # (31)	39 # 37 # (38)	18 # 17 # (18)
	5000	135 # 141 # (138)	18 # 22 # (20)	30 # 26 # (28)	35 # 27 # (31)	10 # 4 # (7)
Positive control not requiring S9Mix	Name	AF-2 #1	NaN_3 #2	AF-2	AF-2	ICR-191 #3
	Concentration (μg/plate)	0.01	0.5	0.01	0.1	1.0
	Number of colonies/plate	623 554 (589)	336 366 (351)	139 149 (144)	479 386 (433)	1057 1138 (1098)
Positive control requiring S9Mix	Name	B[a]P #4	2AA #5	2AA	B[a]P	B[a]P
	Concentration (μg/plate)	5.0	2.0	10.0	5.0	5.0
	Number of colonies/plate	867 819 (843)	181 155 (168)	569 585 (577)	171 160 (166)	125 101 (113)

#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:Benzo[a]pyrene #5:2-Aminoanthracene

#:The test substance was found precipitating on the plates.

Notes:The average number of colonies in each concentration is shown in the ( ).

Fig. 1 No.2862

(Colonies/Plate) D o s e − r e s p o n s e c u r v e (S 9 −)

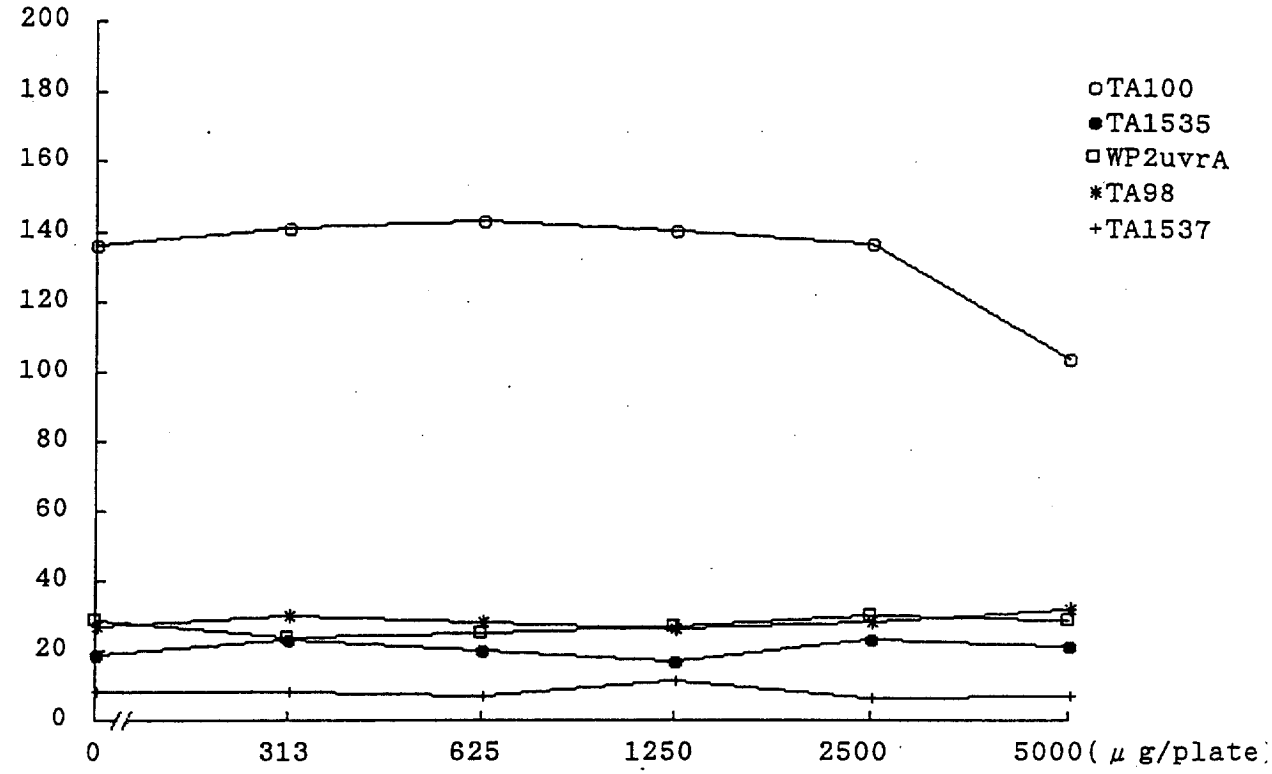


Fig. 2 No.2862

(Colonies/Plate) D o s e − r e s p o n s e c u r v e (S 9 +)