

AR226-297

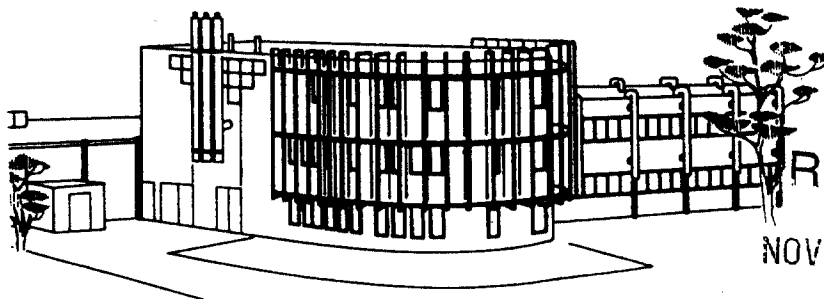


REPORT

EVALUATION OF THE MUTAGENIC ACTIVITY OF
T-5874
IN THE AMES SALMONELLA/MICROSOME TEST
(WITH INDEPENDENT REPEAT)

NOTOX Project 115932
NOTOX Substance 38187

- page 1 of 23 -



RECEIVED

NOV - 9 1994

RM

TOXICOLOGY

004472

Hambakenwetering 3
P.O. Box 3476, 5203 DL 's-Hertogenbosch. The Netherlands
Tel.: 073 - 41 95 75 Fax: 073 - 41 85 43

REPORT

EVALUATION OF THE MUTAGENIC ACTIVITY OF
T-5874
IN THE AMES SALMONELLA/MICROSOME TEST
(WITH INDEPENDENT REPEAT)

NOTOX Project 115932
NOTOX Substance 38187

- page 1 of 23 -

004473

RECEIVED
NOV - 9 1994
2PM
TOXICOLOGY

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

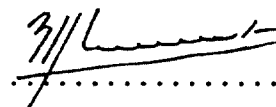
The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

With the exception that the stability of the test substance in the vehicle was unknown.

Study Director

Ing. E.J. van de Waart


.....

Date: 19/04/1994

004474

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands.

Study procedures were subject to periodic inspections and general non study specific processes were also inspected at periodic intervals.

This report was audited by the NOTOX Quality Assurance Unit and the methods and results accurately reflect the raw data.

DATES OF QAU INSPECTIONS/ AUDITS	REPORTING DATES
24-01-1994	24-01-1994
26-01-1994	26-01-1994
15-03-1994	15-03-1994

Quality Assurance Manager

C.J. Mitchell B.Sc.

C.J. Mitchell.....

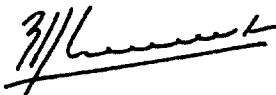
Date: 20-4-94.

004475

REPORT APPROVAL

STUDY DIRECTOR:

Ing. E.J. van de Waart



.....
Date: 19/04/1994

MANAGEMENT:

Dr. I.C. Enninga
Technical Director



.....
Date: 20/04/1994

004476

PREFACE

Sponsor	3M Belgium - Chemical EBC Canadastraat 11 B-2070 ZWIJNDRECHT Belgium
Study Monitor	Mr. R.H. Cox
Testing Facility	NOTOX B.V. Hambakenwetering 3 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Ing. E.J. van de Waart
Technical Coordinator	G. van Oort
Study Plan	Start : January 25, 1994 Completed : February 10, 1994

TEST SUBSTANCE

Identification	T-5874
Description	Cream solid
Batch	2334
Purity	100%
Specific Gravity	1.7
Instructions for test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	January 01, 1996
Stable for at least 4 hours in vehicle	Water : no Dimethylsulphoxide: not indicated

VEHICLE

The test substance was dissolved in dimethylsulphoxide of spectroscopic quality (Merck). Test substance concentrations were prepared directly prior to use.

GUIDELINES

The study procedures described in this report were based on the following guidelines:

- Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Guideline no. 471: "Genetic Toxicology: Salmonella typhimurium Reverse Mutation Assay", (adopted May 26, 1983).
- European Economic Community (EEC), Directive 92/69/EEC. Annex V of the EEC Directive 67/548/EEC, Part B: Methods for the Determination of Toxicity; B.14: "Other Effects-Mutagenicity: Salmonella typhimurium - Reverse Mutation Assay". EEC Publication no. L383 (adopted December, 1992).

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test article reference sample and raw data.

OBJECTIVE

Purpose of the study

The objective of this study was to evaluate the test substance for its ability to induce reverse mutations in a gene of histidine-requiring Salmonella typhimurium bacterial strains to produce histidine-independent strains of these micro-organisms. The assay was conducted in the absence and in the presence of a metabolic system (S9-mix).

Justification for selection of the test system

The Ames test has been shown to be a rapid and inexpensive indicator for the mutagenic activity of a wide range of chemical compounds. The bacterial strains employed were capable of detecting both frameshift and base-pair substitution mutagens.

004478

MATERIALS AND METHODS

TEST SYSTEM

Test System	Salmonella typhimurium bacteria
Rationale	Recognized by the international guidelines as the recommended test system (e.g. EPA, OECD, EEC).
Source	Dr. Bruce N. Ames, University of California at Berkeley, U.S.A. (TA1535, TA1537 and TA98; 1987 and TA100; 1993).

The characteristics of the individual strains were as follows:

<u>Strain</u>	<u>Histidine mutation</u>	<u>Mutation type</u>
TA1537	hisC3076	Frameshift
TA98	hisD3052/R-factor*	Frameshift
TA1535	hisG46	Base-pair substitutions
TA100	hisG46/R-factor*	Base-pair substitutions
*: R-factor = plasmid pKM101 (increases error-prone DNA repair)		

Each tester strain contained the following additional mutations:

rfa : deep rough (defective lipopolysaccharide cellcoat)
gal : mutation in the galactose metabolism
chl : mutation in nitrate reductase
bio : defective biotin synthesis
uvrB: loss of the excision repair system (deletion of the ultraviolet-repair B gene)

Stock cultures of the four strains were stored in liquid nitrogen (-196°C). Strains were regularly checked to confirm their histidine-requirement, crystal violet sensitivity, ampicillin resistance (TA98 and TA100), UV-sensitivity and the number of spontaneous revertants.

CELL CULTURE

Preparation of bacterial cultures

Samples of frozen stock cultures of bacteria were transferred into enriched nutrient broth (Oxoid no. 2) and incubated in a shaking bath (37°C, 150 spm), until the cultures reached an O.D. of 0.4 at 700 nm (10^9 cells/ml). Freshly grown cultures of each strain were used for a test.

004479

Agar plates

Agar plates (ø 9 cm) contained 25 ml glucose agar medium. Glucose agar medium contained per liter: 18 g purified agar (Oxoid, code L28) in Vogel-Bonner Medium E (*), 10 g glucose, 0.5 mg biotin and 0.6 mg histidine.
 (*) Vogel, H.J. and Bonner, D.M., 1956, Acetylornithinase of Escherichia coli: partial purification and some properties. J. Biol. Chem., 218, 97-106.

Top agar

Vogel-Bonner Medium E containing 0.6% (w/v) purified agar was heated to dissolve the agar. Samples of 3 ml top agar were transferred into 10 ml glass tubes with metal caps. Top agar tubes were autoclaved for 20 min at 120°C.

Environmental conditions

All incubations were carried out in the dark at 37°C. The temperature was monitored during the experiment.

REFERENCE SUBSTANCES

Negative control:

The vehicle of the test article.

Positive controls:Without metabolic activation (-S9-mix):

<u>Strain</u>	<u>Chemical</u>	<u>Concentration/plate</u>	<u>Solvent</u>
TA1535	sodium azide (SA) (Fluka)	1 µg	Saline
TA1537	9-aminoacridine (9AC) (Janssen Chimica)	60 µg	Saline
TA98	daunomycine (DM) (Sigma)	4 µg	Saline
TA100	methylmethanesulfonate (MMS) (Merck)	650 µg	DMSO

With metabolic activation (+S9-mix):

<u>Strain</u>	<u>Chemical</u>	<u>Concentration/plate</u>	<u>Solvent</u>
TA1535, TA1537	2-aminoanthracene (2AA)	5 µg	DMSO
TA98, TA100	2-aminoanthracene (2AA) (Sigma)	0.5 µg	DMSO

Solvents for reference substances

Saline = physiological saline (Medital Pharma Ned. B.V.)

DMSO = dimethylsulphoxide of spectroscopic quality (Merck).

604480

METABOLIC ACTIVATION SYSTEM

Preparation of S9-homogenate:

Rat liver microsomal enzymes were routinely prepared from adult male Wistar or Sprague Dawley rats, which were obtained from BRL, Switzerland.

The animals were housed at NOTOX in a special room under standard laboratory conditions, as described in the SOP's. The rats were injected intraperitoneally with a solution (20% w/v) of Aroclor 1254 (500 mg/kg body weight) in corn oil. Five days later, they were killed by decapitation; (they were denied access to food for at least 12 hours preceding sacrifice). The livers of the rats were removed aseptically, and washed in cold (0°C) sterile 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1 mM Na₂-EDTA. Subsequently the livers were minced in a blender and homogenized in 3 volumes of phosphate buffer with a Potter homogenizer. The homogenate was centrifuged for 15 min at 9000 g. The supernatant (S9) was transferred into sterile ampules, which were stored in liquid nitrogen (-196°C).

Preparation of S9-mix:

S9-mix was prepared immediately before use and kept on ice during the test. S9-mix contained per 10 ml: 30 mg NADP and 15.2 mg glucose-6-phosphate in 5.5 ml aqua bidest; 2 ml 0.5 M sodium phosphate buffer pH 7.4; 1 ml 0.08 M MgCl₂ solution; 1 ml 0.33 M KCl solution, and 0.5 ml S9. The above solutions were mixed and filter (0.22 µm)-sterilized (apart from the S9-fraction, which was added after filter-sterilization of the S9-mix components).

EXPERIMENTAL PROCEDURE

Selection of dose levels

Selection of an adequate range of doses was based on a preliminary test with strain TA100, both with and without S9-mix. Eight concentrations were tested in triplicate (this preliminary test was reported as a part of the first experiment of the mutagenesis assay). The highest concentration of test article used in the subsequent mutagenesis assay was 5 mg/plate.

604481

Direct plate incorporation assay *:

Five different doses (with approximately half-log steps) of the test substance were tested in triplicate in each strain. The test substance was tested both with and without S9-mix in each strain, in two independent experiments. Top agar in top agar tubes was melted and heated to 45°C. The following solutions were successively added to 3 ml top agar: 0.1 ml of a fresh bacterial culture (10^9 cells/ml) of one of the tester strains, 0.1 ml of a dilution of the test substance in DMSO, and either 0.5 ml S9-mix (in case of activation assays) or 0.5 ml 0.1 M phosphate buffer (in case of non-activation assays). The ingredients were mixed on a Vortex and the content of the top agar tube was poured onto a selective agar plate. After solidification of the top agar, the plates were turned and incubated in the dark at 37°C for 48 h. After this period revertant (histidine independent) colonies were counted.

- *) 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.
- *) Maron, D.M. and Ames, B.N., 1983, Revised methods for the Salmonella mutagenicity test, Mutation Res., 113, 173-215.
Erratum, 1983, Mutation Res., 113, 533.

Colony counting

The revertant colonies (histidine independent) were counted automatically with an Artek model 880 colony counter or manually, if less than 40 colonies per plate were present. Plates with sufficient test article precipitate to interfere with automated colony counting were counted manually.

ACCEPTABILITY OF ASSAY

An Ames test is considered acceptable if it meets the following criteria:

- a) The negative control data (number of spontaneous revertants per plate) should reasonably be within the laboratory background historical range for each tester strain.
- b) The positive control chemicals should produce responses in all tester strains which also reasonably are within the laboratory historical range documented for each positive control substance.
Furthermore, the mean plate count should be at least two times the concurrent vehicle control group mean.
- c) The selected dose range should include a clearly toxic concentration as demonstrated by the preliminary test with strain TA100 or should extend to 5 mg/plate.

004482

DATA EVALUATION AND STATISTICAL PROCEDURES

No formal hypothesis testing was done.

A test substance is considered negative (not mutagenic) in the Ames test if:

- a) The total number of revertants in any tester strain at any concentration is not greater than two times the solvent control value, with or without metabolic activation.
- b) The negative response should be reproducible in at least one independently repeated experiment.

A test substance is considered positive (mutagenic) in the Ames test if:

- a) It induces at least a 2-fold, dose related increase in the number of revertants with respect to the number induced by the solvent control in any of the tester strains, either with or without metabolic activation. However, any mean plate count of less than 20 is considered to be not significant. If the test substance showed in the first test only a positive response at one or two concentrations, the assay was repeated with doses just below and exceeding those showing positive effects in the first test.
- b) The positive response should be reproducible in at least one independently repeated experiment.

The preceding criteria were not absolute and other modifying factors might enter into the final evaluation decision.

004483

RESULTS

TOXICITY OF THE TEST SUBSTANCE

The survival of the TA100 culture was determined by comparing the number of colonies on the plates containing the test substance with those on the solvent control plates.

Both in the absence and presence of S9-mix the survival of strain TA100 was not reduced up to and including test substance concentrations of 5000 µg/plate (Table 1). Based on these data, the test substance was tested up to and including a concentration of 5000 µg/plate in the absence and presence of S9-mix.

THE AMES SALMONELLA/MICROSOME PLATE TEST

Tables 1 and 2 show the results of the Salmonella/microsome plate test with T-5874. All bacterial strains showed negative responses over the entire dose range of the test substance, i.e. no dose-related, two-fold, increase in the number of revertants in two independently repeated experiments. The negative and strain-specific positive control values were within our laboratory background historical ranges indicating that the test conditions were optimal and that the metabolic activation system functioned properly.

CONCLUSION

Based on the results of this study it is concluded that the test substance is not mutagenic in the Ames Salmonella/microsome assay.

004484

TABLE 1 MUTAGENIC RESPONSE OF T-5874 IN THE AMES SALMONELLA/MICROSOME PLATE TEST.

Experiment 1

Dose (µg/plate)	Mean number of revertant (His ⁺) colonies/ 3 replicate plates ($\pm$ S.D.) with different strains of S.typhimurium			
	TA1535	TA1537	TA98	TA100
<u>Without S9-mix</u>				
3				124 $\pm$ 12
10				129 $\pm$ 7
33				146 $\pm$ 22
100	10 $\pm$ 4	8 $\pm$ 2	24 $\pm$ 4	138 $\pm$ 32
333	8 $\pm$ 3	7 $\pm$ 3	26 $\pm$ 3	149 $\pm$ 17
1000	8 $\pm$ 5	9 $\pm$ 2	23 $\pm$ 2	138 $\pm$ 11
3330 ²	10 $\pm$ 3	8 $\pm$ 3	22 $\pm$ 2	146 $\pm$ 15
5000 ²	10 $\pm$ 2	8 $\pm$ 3	22 $\pm$ 3	144 $\pm$ 14
Solvent control ¹	6 $\pm$ 2	7 $\pm$ 3	21 $\pm$ 2	163 $\pm$ 11
Positive control	274 $\pm$ 28	577 $\pm$ 89	643 $\pm$ 54	1372 $\pm$ 29
<u>With S9-mix</u>				
3				141 $\pm$ 8
10				143 $\pm$ 4
33				130 $\pm$ 10
100	9 $\pm$ 2	7 $\pm$ 3	23 $\pm$ 4	136 $\pm$ 7
333	11 $\pm$ 2	6 $\pm$ 4	25 $\pm$ 2	147 $\pm$ 7
1000	8 $\pm$ 4	6 $\pm$ 3	28 $\pm$ 3	140 $\pm$ 5
3330 ²	5 $\pm$ 5	6 $\pm$ 3	24 $\pm$ 5	150 $\pm$ 19
5000 ²	7 $\pm$ 3	7 $\pm$ 1	25 $\pm$ 4	147 $\pm$ 8
Solvent control ¹	7 $\pm$ 2	8 $\pm$ 4	25 $\pm$ 3	170 $\pm$ 11
Positive control	218 $\pm$ 34	665 $\pm$ 86	253 $\pm$ 7	601 $\pm$ 31

¹ 0.1 ml DMSO² Test substance precipitated slightly on the plates

004485

TABLE 2 MUTAGENIC RESPONSE OF T-5874 IN THE AMES SALMONELLA/MICROSOME PLATE TEST.

Experiment 2

Dose (μ g/plate)	Mean number of revertant (His ⁺) colonies/ 3 replicate plates ($\pm$ S.D.) with different strains of S.typhimurium			
	TA1535	TA1537	TA98	TA100
<u>Without S9-mix</u>				
100	7 $\pm$ 3	8 $\pm$ 2	30 $\pm$ 2	108 $\pm$ 18
333	10 $\pm$ 1	8 $\pm$ 2	28 $\pm$ 7	126 $\pm$ 3
1000	8 $\pm$ 2	6 $\pm$ 3	29 $\pm$ 1	124 $\pm$ 10
3330 ²	21 $\pm$ 10	6 $\pm$ 1	29 $\pm$ 3	161 $\pm$ 6
5000 ²	17 $\pm$ 4	12 ; 15 ³	25 $\pm$ 5	129 $\pm$ 6
Solvent control ¹	13 $\pm$ 4	4 $\pm$ 2	28 $\pm$ 1	123 $\pm$ 7
Positive control	257 $\pm$ 13	391 $\pm$ 45	477 $\pm$ 69	1292 $\pm$ 59
<u>With S9-mix</u>				
100	11 $\pm$ 4	5 $\pm$ 3	34 $\pm$ 13	120 $\pm$ 6
333	6 $\pm$ 4	5 $\pm$ 4	30 $\pm$ 5	118 $\pm$ 15
1000	7 $\pm$ 3	7 $\pm$ 2	36 $\pm$ 8	133 $\pm$ 6
3330 ²	11 $\pm$ 3	6 $\pm$ 1	40 $\pm$ 12	114 $\pm$ 17
5000 ²	5 $\pm$ 2	7 $\pm$ 2	31 $\pm$ 5	143 $\pm$ 2
Solvent control ¹	5 $\pm$ 1	4 $\pm$ 2	26 $\pm$ 7	114 $\pm$ 3
Positive control	174 $\pm$ 55	216 $\pm$ 56	339 $\pm$ 23	649 $\pm$ 56

¹ 0.1 ml DMSO² Test substance precipitated slightly on the plates³ One plate infected with other bacteria

004486

APPENDIX 1

Evidence of test article precipitate on the plates is recorded by addition of the following precipitation code.

PRECIPITATION CODE

Definition	Characteristics
Slight Precipitate	Distinguished by noticeable precipitate on the plate. However, the precipitate does not influence automated counting of the plate.
Moderate Precipitate	Distinguished by a marked amount of precipitate on the plate, requiring the plate to be hand counted.
Heavy Precipitate	Distinguished by a large amount of precipitate on the plate, making the required hand count difficult.

004487

APPENDIX 2

Individual plate counts; (following pages)

Strain TA1535
Experiment 1

WITHOUT S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	11	5	13	10 $\pm$	4
333	6	8	11	8 $\pm$	3
1000	12	3	8	8 $\pm$	5
3330 ¹	13	9	7	10 $\pm$	3
5000 ¹	9	13	9	10 $\pm$	2
Solvent control	8	4	7	6 $\pm$	2
Positive control	296	283	242	274 $\pm$	28

WITH S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	12	8	8	9 $\pm$	2
333	11	13	9	11 $\pm$	2
1000	5	7	12	8 $\pm$	4
3330 ¹	1	10	5	5 $\pm$	5
5000 ¹	5	5	11	7 $\pm$	3
Solvent control	9	5	8	7 $\pm$	2
Positive control	185	217	253	218 $\pm$	34

¹ Test substance precipitated slightly on the plates

004488

Strain TA1537
Experiment 1

WITHOUT S9-MIX

plate	1	2	3	MEAN	SD
Dose(µg/plate)					
100	6	10	7	8 ±	2
333	4	9	9	7 ±	3
1000	10	11	7	9 ±	2
3330 ¹	9	10	4	8 ±	3
5000 ¹	6	11	8	8 ±	3
Solvent control	5	11	6	7 ±	3
Positive control	679	516	535	577 ±	89

WITH S9-MIX

plate	1	2	3	MEAN	SD
Dose(µg/plate)					
100	5	5	10	7 ±	3
333	6	9	2	6 ±	4
1000	5	9	4	6 ±	3
3330 ¹	3	7	8	6 ±	3
5000 ¹	7	8	7	7 ±	1
Solvent control	3	11	10	8 ±	4
Positive control	645	760	591	665 ±	86

¹ Test substance precipitated slightly on the plates

004489

Strain TA98
Experiment 1

WITHOUT S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	22	29	21	24 $\pm$	4
333	29	26	23	26 $\pm$	3
1000	25	23	22	23 $\pm$	2
3330 ¹	20	22	23	22 $\pm$	2
5000 ¹	24	19	23	22 $\pm$	3
Solvent control	22	19	21	21 $\pm$	2
Positive control	696	588	644	643 $\pm$	54

WITH S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	19	27	24	23 $\pm$	4
333	26	22	26	25 $\pm$	2
1000	31	28	25	28 $\pm$	3
3330 ¹	23	29	20	24 $\pm$	5
5000 ¹	27	28	21	25 $\pm$	4
Solvent control	28	23	23	25 $\pm$	3
Positive control	260	246	254	253 $\pm$	7

¹ Test substance precipitated slightly on the plates

004490

Strain TA100
Experiment 1

WITHOUT S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
3	125	112	136	124 $\pm$ 12	
10	123	129	136	129 $\pm$ 7	
33	157	160	120	146 $\pm$ 22	
100	101	158	154	138 $\pm$ 32	
333	134	145	168	149 $\pm$ 17	
1000	138	148	127	138 $\pm$ 11	
3330 ¹	149	159	130	146 $\pm$ 15	
5000 ¹	158	131	144	144 $\pm$ 14	
Solvent control	150	168	170	163 $\pm$ 11	
Positive control	1383	1339	1395	1372 $\pm$ 29	

WITH S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
3	143	133	148	141 $\pm$ 8	
10	144	146	138	143 $\pm$ 4	
33	140	128	121	130 $\pm$ 10	
100	137	142	128	136 $\pm$ 7	
333	150	139	153	147 $\pm$ 7	
1000	146	138	136	140 $\pm$ 5	
3330 ¹	130	151	168	150 $\pm$ 19	
5000 ¹	139	155	148	147 $\pm$ 8	
Solvent control	162	182	166	170 $\pm$ 11	
Positive control	637	586	581	601 $\pm$ 31	

¹ Test substance precipitated slightly on the plates

004491

Strain TA1535
Experiment 2

WITHOUT S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	11	6	5	7 $\pm$	3
333	10	9	10	10 $\pm$	1
1000	8	7	10	8 $\pm$	2
3330 ¹	17	33	14	21 $\pm$	10
5000 ¹	21	16	14	17 $\pm$	4
Solvent control	13	17	9	13 $\pm$	4
Positive control	245	256	270	257 $\pm$	13

WITH S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	7	14	11	11 $\pm$	4
333	3	11	5	6 $\pm$	4
1000	4	10	8	7 $\pm$	3
3330 ¹	8	11	13	11 $\pm$	3
5000 ¹	4	5	7	5 $\pm$	2
Solvent control	4	5	6	5 $\pm$	1
Positive control	111	215	196	174 $\pm$	55

¹ Test substance precipitated slightly on the plates

004492

Strain TA1537
Experiment 2

WITHOUT S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	8	10	6	8 $\pm$	2
333	7	7	10	8 $\pm$	2
1000	6	8	3	6 $\pm$	3
3330 ¹	5	6	7	6 $\pm$	1
5000 ¹	INF	12	15	12 ;	15
Solvent control	4	2	6	4 $\pm$	2
Positive control	441	355	378	391 $\pm$	45

WITH S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	7	2	5	5 $\pm$	3
333	9	2	5	5 $\pm$	4
1000	7	8	5	7 $\pm$	2
3330 ¹	5	6	7	6 $\pm$	1
5000 ¹	4	8	8	7 $\pm$	2
Solvent control	4	6	2	4 $\pm$	2
Positive control	276	208	165	216 $\pm$	56

¹ Test substance precipitated slightly on the plates
INF = Plate infected with other bacteria

004493

Strain TA98
Experiment 2

WITHOUT S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	30	31	28	30 $\pm$	2
333	30	34	21	28 $\pm$	7
1000	30	29	29	29 $\pm$	1
3330 ¹	26	32	30	29 $\pm$	3
5000 ¹	31	24	21	25 $\pm$	5
Solvent control	27	29	28	28 $\pm$	1
Positive control	437	557	438	477 $\pm$	69

WITH S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	29	25	49	34 $\pm$	13
333	25	35	30	30 $\pm$	5
1000	43	38	28	36 $\pm$	8
3330 ¹	31	36	53	40 $\pm$	12
5000 ¹	36	27	30	31 $\pm$	5
Solvent control	18	30	31	26 $\pm$	7
Positive control	314	343	360	339 $\pm$	23

¹ Test substance precipitated slightly on the plates

004494

Strain TA100
Experiment 2

WITHOUT S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	110	124	89	108 $\pm$	18
333	123	126	128	126 $\pm$	3
1000	114	125	134	124 $\pm$	10
3330 ¹	168	160	156	161 $\pm$	6
5000 ¹	128	135	123	129 $\pm$	6
Solvent control	124	129	115	123 $\pm$	7
Positive control	1252	1360	1265	1292 $\pm$	59

WITH S9-MIX

plate	1	2	3	MEAN	SD
Dose(μ g/plate)					
100	118	116	127	120 $\pm$	6
333	104	134	115	118 $\pm$	15
1000	133	138	127	133 $\pm$	6
3330 ¹	100	133	110	114 $\pm$	17
5000 ¹	141	143	144	143 $\pm$	2
Solvent control	114	111	116	114 $\pm$	3
Positive control	585	677	685	649 $\pm$	56

¹ Test substance precipitated slightly on the plates

004495