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July 2, 1998

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Dear Sir/Madam:

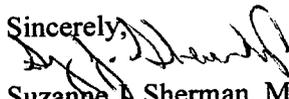
The purpose of this letter is to inform you under Section 8(e) of TSCA of the preliminary results recently obtained from a study entitled "AERO® 5100 Promoter - Bacterial Mutation Assay" conducted with a commercial material which contained ~98% Carbamothioic acid, 2-propenyl-, O-(2-methylpropyl) ester [CAS Number 86329-09-1].

The contract laboratory has issued a draft report, which showed clear evidence of mutagenic activity in strain TA1535, in the presence of S9 mix, in a dose responsive fashion. In addition, small increases (<2 fold) in revertant colony numbers were also obtained in strain TA100 in the presence of S9 mix.

Once the final report is received, we will forward a copy of the report for your reference.

Please direct all communications on this subject to Patricia Ann Vernon, Product Regulatory Compliance Manager, Asia-Pacific at the address above or call her at (973) 357-3375.

Sincerely,


Suzanne A. Sherman, M.D.
Corporate Medical Director

Cc: F. Cappuccitti - CF
R. Deskin
K. E. Koster
D. Monaghan
R. Ryles - ST

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Huntingdon Life Sciences

**AERO® 5100 PROMOTER
BACTERIAL MUTATION ASSAY**

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Report

**AERO® 5100 PROMOTER
BACTERIAL MUTATION ASSAY**

DRAFT

Sponsor

**Cytec Industries Inc.,
Five Garret Mountain Plaza,
West Paterson,
N J 07424,
USA**

Research Laboratory

**Huntingdon Life Sciences Ltd.,
Eye,
Suffolk,
IP23 7PX,
ENGLAND**

Draft report issued 17 June 1998

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards, with the exception stated below, and I consider the data generated to be valid.

The United Kingdom Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654).

EC Council Directive 87/18 EEC of 18 December 1986 (Official Journal No L 15/29).

Good Laboratory Practice in the Testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished in OECD Environment Monograph No 45 1992 and subsequently OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

In line with normal practice in this type of short-term study, the protocol did not require analysis of the dose form.

.....
Kenneth May, B.Sc., C.Biol., M.I.Biol.,
Study Director,
Huntingdon Life Sciences Ltd.

.....
Date

QUALITY ASSURANCE STATEMENT

This report has been audited by the Department of Quality Assurance, Huntingdon Life Sciences Ltd. The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon Life Sciences during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon Life Sciences.

Certain studies, such as that described in this report, are conducted at Huntingdon Life Sciences in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and/or Huntingdon Life Sciences Management as appropriate.

Date(s) of inspection
Protocol check
S9 preparation
Audit
Formulation
Plate scoring
Treatment

Date(s) of reporting inspection findings to the
Study Director and/or Huntingdon Life Sciences Management
Protocol check
S9 preparation
Audit
Formulation
Plate scoring
Treatment

Date of reporting audit findings (review of the final report) to the
Study Director and/or Huntingdon Life Sciences Management

.....
Dawn Chase, M.I.A.T.,
Principal Auditor,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

.....
Date

RESPONSIBLE PERSONNEL

**Kenneth May, B.Sc., C.Biol., M.I.Biol.,
Senior Genetic Toxicologist,
Cellular Toxicology**

**John Kernahan, B.Sc.,
Scientific Officer,
Cellular Toxicology**

SUMMARY

In this *in vitro* assessment of the mutagenic potential of AERO® 5100 Promoter, histidine dependent auxotrophic mutants of *Salmonella typhimurium*, strains TA1535, TA1537, TA98 and TA100, and a tryptophan dependent mutant of *Escherichia coli*, strain CM891 (WP2uvrA/pKM101), were exposed to the test substance diluted in ethanol, which was also used as a negative control.

Two independent mutation tests were performed in the presence and absence of liver preparations from Aroclor 1254-induced rats (S9 mix). Both were standard plate incorporation assays, but in the second, the proportion of S9 fraction in the S9 mix was increased from 10% to 20%.

Concentrations of up to 5000 µg/plate were tested in the mutation tests. This is the standard limit concentration recommended in the regulatory guidelines this assay follows. Other concentrations used were a series of *ca* half-log₁₀ dilutions of the highest concentration.

Inhibition of bacterial growth, observed as thinning of the background lawn of non-revertant cells and reduction in revertant colony numbers, occurred in all strains following exposure to AERO® 5100 Promoter at 5000 µg/plate.

Clear evidence of mutagenic activity was seen in strain TA1535 in both tests, in the presence of S9 mix, following exposure to AERO® 5100 Promoter. Reversion to prototrophy was maximal at 1500 µg/plate with a clear dose-response relationship.

Small increases (< 2- fold) in revertant colony numbers over control counts were also obtained in strain TA100 in the presence of S9 mix following exposure to AERO® 5100 Promoter. More substantial (> 2- fold) increases, were observed in the presence of "double-strength" S9 mix in the second test. Reversion to prototrophy was maximal at 1500 µg/plate with a clear dose-response relationship.

The concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the liver preparations.

It is concluded that, when tested in ethanol, AERO® 5100 Promoter shows evidence of mutagenic activity in strains of *S. typhimurium* capable of detecting base-pair substitutions, with a clear requirement for metabolic activation.

INTRODUCTION

This report describes a study designed to assess the mutagenic potential of AERO® 5100 Promoter in a bacterial system. The study was conducted in compliance with the following guidelines:

OECD Guidelines for Testing of Chemicals. (1997) No. 471: Genetic Toxicology: Bacterial Reverse Mutation Test.

EEC Annex to Directive 92/69/EEC. (1992) Part B : Methods for Determination of Toxicity, B.13. Other effects - Mutagenicity: *Escherichia coli* - Reverse Mutation Assay. O.J. No. L 383 A, 157.

EEC Annex to Directive 92/69/EEC. (1992) Part B : Methods for Determination of Toxicity, B.14. Other effects - Mutagenicity: *Salmonella typhimurium* - Reverse Mutation Assay. O.J. No. L 383 A, 160.

US EPA 40 CFR Part 799 (1997) Toxic Substances Control Act Test Guidelines - Sub-section 799.9510, TSCA bacterial reverse mutation test. Federal Register, Vol. 62, No. 158.

The method described was also designed to comply with ICH (1996 & 1997), and followed the recommendations of the United Kingdom Environmental Mutagen Society (Gatehouse *et al* 1990).

The *in vitro* technique described by Ames and his co-workers, (Ames, McCann and Yamasaki 1975, Maron and Ames 1983) enables the mutagenic effect of a test substance to be determined by exposing specially selected strains of *Salmonella typhimurium* to the test substance. Normally *S. typhimurium* is capable of synthesising the essential amino acid, histidine, but the mutant strains used in this test are incapable of this function. When these strains are exposed to a mutagen, reverse mutation to the original histidine independent form takes place in a proportion of the population. These are referred to as revertants, and are readily detected by their ability to grow and form colonies on a histidine deficient medium (supplemented with biotin, since these strains are also incapable of biotin synthesis).

A technique based on similar principles has also been described by Green (1984). This system employs mutant strains of *Escherichia coli* which are incapable of synthesising the amino acid tryptophan required for growth.

The strains used carry additional mutations which render them more sensitive to mutagens. The *S. typhimurium* strains have a defective cell coat which allows greater permeability of test substances into the cell. All the strains are deficient in normal DNA repair processes. In addition three of them possess a plasmid (pKM101) which introduces an error-prone repair process, resulting in increased sensitivity to some mutagens.

Many substances do not exert a mutagenic effect until they have been metabolised by enzyme systems not available in the bacterial cell. Therefore the bacteria and test substance are incubated in both the absence and presence of a supplemented liver fraction (S9 mix) prepared from rats previously treated with a substance (Aroclor 1254) known to induce a high level of enzymic activity.

The protocol was approved by Huntingdon Life Sciences Management on 2 April 1998, the Sponsor on 14 April 1998 and by the Study Director on 1 June 1998.

The experimental phase of the study was conducted between 1 and 8 June 1998.

TEST SUBSTANCE

Identity: AERO® 5100 Promoter

Alternative name: CT-637-97

Chemical name: Carbamothioic acid,
2-propenyl-, O-(2-methylpropyl) ester

CAS number: 86329-09-1

Appearance: Pale brown liquid

Storage conditions: Room temperature, dark

Lot number: 95

Expiry date: March 1999

Purity: ~ 98%

Date received: 4 March 1998

EXPERIMENTAL PROCEDURE

BACTERIAL STRAINS

The following strains were used:-

S. typhimurium TA1535: contains a histidine missense mutation (*hisG46*) but is also deficient in a DNA repair system (*uvrB*) and has a defective lipopolysaccharide coat on the cell wall (*rfa* mutation). It is reverted by many agents causing base-pair substitutions, but is not sensitive to frameshift mutagens.

S. typhimurium TA100: is the same as TA1535 but contains a resistance transfer factor conferring ampicillin resistance and increasing sensitivity to some mutagens (plasmid pKM101). In addition to base-pair substitutions, it is also able to detect certain frameshift mutagens.

S. typhimurium TA1537: bears a histidine frameshift mutation (*hisC3076*). Like TA1535, it is defective in a DNA repair system and lipopolysaccharide coat. It is sensitive to agents causing frameshift mutations involving insertion or deletion of a single base-pair.

S. typhimurium TA98: contains another histidine frameshift mutation (*hisD3052*). Again it has a defective DNA repair system and lipopolysaccharide coat but also contains the pKM101 plasmid. It is reverted by agents causing deletion of two adjacent base-pairs (double frameshift mutations), but not by simple alkylating agents causing base-pair substitutions.

E. coli CM891: (WP2uvrA/pKM101) contains an ochre mutation. It is reverted by many agents causing A-T base-pair substitutions at the *trpE* locus or by G-C base-pair substitutions in transfer RNA loci elsewhere in the chromosome. It is also deficient in a DNA repair system (*uvrA*), and is more readily reverted by certain mutagens than its parent strain WP2. It also contains the pKM101 plasmid.

The strains of *S. typhimurium* were obtained from Professor B.N. Ames, University of California, Berkeley, California, USA.

The strain of *E. coli* was obtained from the National Collections of Industrial and Marine Bacteria, Aberdeen, Scotland.

Batches of the strains were obtained from master stocks held in liquid nitrogen. The test batches were aliquots of nutrient broth cultures and were stored at -80°C. Dimethyl sulphoxide (DMSO) was added to the cultures at 8% v/v as a cryopreservative. Each batch of frozen strain was tested, where applicable, for cell membrane permeability (*rfa* mutation), sensitivity to UV light and the pKM101 plasmid which confers resistance to ampicillin. The responses of the strains to a series of diagnostic mutagens was also assessed.

For use in tests an aliquot of frozen culture was added to 25 ml of nutrient broth (Merck No. 2) and incubated, with shaking, at 37°C for 10 hours. These cultures provided at least 2×10^9 cells per ml which were measured by spreading aliquots (0.1 ml) of a 10^{-6} dilution of the overnight cultures on the surface of plates of nutrient agar.

POSITIVE CONTROLS

In the absence of S9 mix

Identity: *N*-Ethyl-*N'*-nitro-*N*-nitrosoguanidine
Supplier: Sigma Chemical
Lot number: 20F-0235
Purity: $\geq 97\%$
Appearance: Pale yellow crystalline powder
Solvent: DMSO (Aldrich, A.C.S. spectrophotometric grade, $\geq 99.9\%$ pure)
Concentration: 5 $\mu\text{g}/\text{plate}$ for strain TA1535
3 $\mu\text{g}/\text{plate}$ for strain TA100
2 $\mu\text{g}/\text{plate}$ for strain CM891

Identity: 9-Aminoacridine
Supplier: Sigma Chemical
Batch number: 108C-0358
Purity: 99%
Appearance: Yellow powder
Solvent: DMSO (Aldrich, A.C.S. spectrophotometric grade, $\geq 99.9\%$ pure)
Concentration: 80 $\mu\text{g}/\text{plate}$ for strain TA1537

Identity: 2-Nitrofluorene
Supplier: Aldrich Chemical Company
Batch number: 012867
Purity: 98%
Appearance: Beige powder
Solvent: DMSO (Aldrich, A.C.S. spectrophotometric grade, $\geq 99.9\%$ pure)
Concentration: 1 $\mu\text{g}/\text{plate}$ for strain TA98

In the presence of S9 mix

Identity: 2-Aminoanthracene
Supplier: Aldrich Chemical Company
Batch number: 52234-024
Purity: 96%
Appearance: Green powder
Solvent: DMSO (Aldrich, A.C.S. spectrophotometric grade, $\geq 99.9\%$ pure)
Concentration: 2 $\mu\text{g}/\text{plate}$ for strain TA1535
10 $\mu\text{g}/\text{plate}$ for strain CM891

Identity: Benzo[a]pyrene
Supplier: Aldrich Chemical Company
Batch number: 07778-105
Purity: 98%
Appearance: Yellow powder
Solvent: DMSO (Aldrich, A.C.S. spectrophotometric grade, $\geq 99.9\%$ pure)
Concentration: 5 $\mu\text{g}/\text{plate}$ for strains TA1537, TA98 and TA100

PREPARATION OF S9 FRACTION

Species:	Rat
Sex:	Male
Strain:	Sprague-Dawley derived
Source:	Harlan Olac Ltd
Age:	7-8 weeks
Weight:	<300 g

S9 fraction was prepared from a group of *ca* 10 animals. Mixed function oxidase systems in the rat livers were stimulated by Aroclor 1254, administered as a single intra-peritoneal injection in Arachis oil at a dosage of 500 mg/kg bodyweight. On the fifth day after injection, following an overnight starvation, the rats were killed and their livers aseptically removed.

The following steps were carried out at 0-4°C under aseptic conditions. The livers were placed in 0.15 M KCl (3 ml KCl : 1 g liver) before being transferred to an Ultra-Turrax homogeniser. Following preparation, the homogenate was centrifuged at 9000 g for 10 minutes. The supernatant fraction (S9 fraction) was dispensed into aliquots and stored at -80°C until required. The efficacy of each batch of S9 fraction was tested in a bacterial mutation assay with the mutagenic precursors 7,12-dimethylbenzanthracene and 2-aminoanthracene before use. The sterility was also checked.

Date of preparation:	11 March 1998	test 1
	23 April 1998	test 2

PREPARATION OF S9 MIX

S9 mix contained: S9 fraction (10% v/v for test 1; 20% v/v for test 2), MgCl₂ (8 mM), KCl (33 mM), sodium orthophosphate buffer pH 7.4 (100 mM), glucose-6-phosphate (5 mM), NADP (4 mM). All the cofactors were filter-sterilised before use.

SELECTION OF SOLVENT AND FORMULATION OF TEST SUBSTANCE

Prior to commencing testing, the solubility of the test substance was assessed at 50 mg/ml in ethanol, in which it dissolved. Therefore ethanol (Fisher, analytical reagent grade, lot No. 9629 150386, 99.8% pure) was used as the solvent for this study.

All concentrations cited in this report are expressed in terms of the AERO® 5100 Promoter sample as received.

MUTATION TEST PROCEDURE

First test

The test substance was added to cultures of the five tester strains at seven concentrations separated by *ca* half-log₁₀ intervals. The highest concentration of AERO® 5100 Promoter tested was 50 mg/ml in the chosen solvent, which provided a final concentration of 5000 µg/plate. This is the standard limit concentration recommended in the regulatory guidelines this assay follows. The negative control was the chosen solvent, ethanol. The appropriate positive controls were also included.

An aliquot of 0.1 ml of a 10 hour bacterial culture and 0.5 ml S9 mix or 0.5 ml 0.1 M phosphate buffer (pH 7.4) were placed in glass tubes. An aliquot of 0.1 ml of the test solution was added, followed immediately by 2 ml of molten agar containing 0.5mM histidine/biotin/tryptophan. The mixture was thoroughly shaken and overlaid onto previously prepared petri dishes containing 25 ml minimal agar. Each petri dish was individually labelled with a unique code corresponding to a sheet, identifying the dish's contents. Three petri dishes were used for each concentration. Plates were also prepared without the addition of bacteria in order to assess the sterility of the test substance, S9 mix and phosphate buffer. All plates were incubated at 37°C for *ca* 72 hours. After this period the appearance of the background bacterial lawn was examined and revertant colonies counted using a Domino automated colony counter.

Any toxic effects of the test substance would be detected by a substantial reduction in revertant colony counts or by the absence of a complete background bacterial lawn. In the absence of any toxic effects the top concentration normally used in the second test would be the same as that used in the first. If toxic effects were observed a lower concentration may be chosen. It should be ensured that if a lower concentration was chosen, signs of bacterial inhibition are present at the top concentration. Ideally a minimum of three non-toxic concentrations should be obtained.

Second test

A variation to the test procedure was used for the second test to try to elicit a less equivocal result in strain TA100. The variation was to increase the proportion of S9 fraction in the S9 mix from 10% to 20%. This modification of the test method was employed as an alternative to the inclusion of a pre-incubation stage in order to avoid possible toxic effects of ethanol. 5000 µg/plate was again chosen as the top concentration, but only five concentrations were used.

STABILITY AND FORMULATION ANALYSIS

The stability of the test substance and of the test substance in the solvent were not determined as part of this study. Analysis of achieved concentration was not performed as part of this study.

ASSESSMENT OF RESULTS

For a test to be considered valid the mean of the solvent control revertant colony numbers for each strain should lie in the range stated in the appropriate Standard Operating Procedure. Also, the positive control compounds must cause at least a doubling of mean revertant colony numbers over the negative control.

The mean number of revertant colonies for all treatment groups were compared with those obtained for the solvent control groups. The mutagenic activity of a test substance was assessed by applying the following criteria:

- a) If treatment with a test substance produces an increase in revertant colony numbers of at least twice the concurrent solvent controls, with some evidence of a positive dose-relationship, in two separate experiments, with any bacterial strain either in the presence or absence of S9 mix, it is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.
- b) If treatment with a test substance does not produce reproducible increases of at least 1.5 times the concurrent solvent controls in either mutation test it is considered to show no evidence of mutagenic activity in this test system. No statistical analysis is performed.
- c) If the results obtained fail to satisfy the criteria for a clear "positive" or "negative" response given in paragraphs a) and b), additional testing may be performed in order to resolve the issue of the test substance's mutagenic activity in this test system. Should an increase in revertant colony numbers then be observed which satisfies paragraph (a) the substance is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

If no clear "positive" response can be obtained, the test data may be subjected to analysis to determine the statistical significance of any observed increases in revertant colony numbers. The statistical procedures used will be those described by Mahon *et al* (1989) and will usually be analysis of variance followed by Dunnett's test.

MAINTENANCE OF RECORDS

All experimental data arising from the study (including documentary raw data, records and other materials; collectively defined as the "materials") will remain the property of the Sponsor.

Huntingdon Life Sciences shall retain the materials in its archive at Eye for a period of five years from the date of issue of the final report. After such time, the Sponsor will be contacted and their advice sought on the return, disposal or further retention of the materials. If requested, Huntingdon Life Sciences will continue to retain the materials, subject to a reasonable fee being agreed with the Sponsor.

Huntingdon Life Sciences shall also retain a copy of the final report in its archive at Eye indefinitely.

RESULTS

The results obtained with AERO® 5100 Promoter and positive control compounds are presented in Tables 1 to 5. The mean values quoted have been corrected to the nearest whole number.

The absence of colonies on sterility check plates confirmed the absence of microbial contamination.

The total colony counts on nutrient agar plates (see Tables) confirmed the viability and high cell density of the cultures of the individual organisms.

The mean revertant colony counts for the solvent controls were within the ranges stated in the appropriate Standard Operating Procedure. Appropriate positive control chemicals (with S9 mix where required) induced substantial increases in revertant colony numbers with all strains, confirming sensitivity of the cultures and activity of the S9 mix.

FIRST TEST

Substantial increases in revertant colony numbers over control counts were obtained with strain TA1535 in the presence of S9 mix following exposure to AERO® 5100 Promoter. Small increases (< 2- fold) in revertant colony numbers over control counts were also obtained in strain TA100 in the presence of S9 mix following exposure to AERO® 5100 Promoter. In both strains increases were maximal at 1500 µg/plate and a clear dose-response relationship was observed.

Inhibition of bacterial growth, observed as thinning of the background lawn of non-revertant cells and reduction in revertant colony numbers, occurred in all strains following exposure to AERO® 5100 Promoter at 5000 µg/plate.

SECOND TEST

Substantial increases in revertant colony numbers over control counts were obtained with strains TA1535 and TA100 in the presence of S9 mix following exposure to AERO® 5100 Promoter. Increases were maximal at 1500 µg/plate and a clear dose-response relationship was observed.

Inhibition of bacterial growth, observed as thinning of the background lawn of non-revertant cells and reduction in revertant colony numbers, occurred in all strains following exposure to AERO® 5100 Promoter at 5000 µg/plate.

CONCLUSION

It is concluded that, when tested in ethanol, AERO® 5100 Promoter shows evidence of mutagenic activity in strains of *S. typhimurium* capable of detecting base-pair substitutions, with a clear requirement for metabolic activation.

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TABLE 1

Results obtained with *Salmonella typhimurium* TA98 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 1				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	22 t	19 t	19 t	20	2
4	AERO® 5100 Promoter (1500)	+	39	30	34	34	5
5	AERO® 5100 Promoter (500)	+	39	38	34	37	3
6	AERO® 5100 Promoter (150)	+	41	41	31	38	6
7	AERO® 5100 Promoter (50)	+	37	37	36	37	1
8	AERO® 5100 Promoter (15)	+	41	39	39	40	1
9	AERO® 5100 Promoter (5)	+	49	45	35	43	7
10	Ethanol (0.1 ml)	+	38	39	38	38	1
11	AERO® 5100 Promoter (5000)	-	22 t	19 t	19 t	20	2
12	AERO® 5100 Promoter (1500)	-	37	32	28	32	5
13	AERO® 5100 Promoter (500)	-	43	41	32	39	6
14	AERO® 5100 Promoter (150)	-	39	34	51	41	9
15	AERO® 5100 Promoter (50)	-	31	28	38	32	5
16	AERO® 5100 Promoter (15)	-	34	51	46	44	9
17	AERO® 5100 Promoter (5)	-	36	29	43	36	7
18	Ethanol (0.1 ml)	-	39	39	41	40	1
19	Benzo[a]pyrene (5)	+	654	720	682	685	33
20	2-Nitrofluorene (1)	-	249	242	328	273	48
21	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	95	100	103	99	4
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

TABLE 1 - continued

Results obtained with *Salmonella typhimurium* TA98 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 2				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	15 t	9 t	8 t	11	4
4	AERO® 5100 Promoter (1500)	+	35	35	28	33	4
5	AERO® 5100 Promoter (500)	+	35	41	31	36	5
6	AERO® 5100 Promoter (150)	+	32	43	43	39	6
7	AERO® 5100 Promoter (50)	+	28	34	42	35	7
8	Ethanol (0.1 ml)	+	38	38	37	38	1
9	AERO® 5100 Promoter (5000)	-	13 t	8 t	7 t	9	3
10	AERO® 5100 Promoter (1500)	-	34	38	29	34	5
11	AERO® 5100 Promoter (500)	-	32	37	38	36	3
12	AERO® 5100 Promoter (150)	-	45	41	38	41	4
13	AERO® 5100 Promoter (50)	-	45	39	36	40	5
14	Ethanol (0.1 ml)	-	43	34	38	38	5
15	Benzo[a]pyrene (5)	+	522	466	502	497	28
16	2-Nitrofluorene (1)	-	225	225	234	228	5
17	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	110	106	100	105	5
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

TABLE 2

Results obtained with *Salmonella typhimurium* TA100 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 1				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	68 t	55 t	46 t	56	11
4	AERO® 5100 Promoter (1500)	+	170	169	175	171	3
5	AERO® 5100 Promoter (500)	+	165	165	164	165	1
6	AERO® 5100 Promoter (150)	+	132	133	157	141	14
7	AERO® 5100 Promoter (50)	+	114	116	118	116	2
8	AERO® 5100 Promoter (15)	+	101	95	95	97	3
9	AERO® 5100 Promoter (5)	+	111	92	96	100	10
10	Ethanol (0.1 ml)	+	96	94	101	97	4
11	AERO® 5100 Promoter (5000)	-	63 t	56 t	67 t	62	6
12	AERO® 5100 Promoter (1500)	-	108	94	79	94	15
13	AERO® 5100 Promoter (500)	-	85	95	86	89	6
14	AERO® 5100 Promoter (150)	-	81	95	92	89	7
15	AERO® 5100 Promoter (50)	-	102	97	88	96	7
16	AERO® 5100 Promoter (15)	-	97	106	88	97	9
17	AERO® 5100 Promoter (5)	-	86	95	86	89	5
18	Ethanol (0.1 ml)	-	86	114	93	98	15
19	Benzo[a]pyrene (5)	+	496	462	518	492	28
20	ENNG (3)	-	449	414	449	437	20
21	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	116	111	114	114	3
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

TABLE 2 - continued

Results obtained with *Salmonella typhimurium* TA100 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 2				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	41 t	49 t	49 t	46	5
4	AERO® 5100 Promoter (1500)	+	228	218	187	211	21
5	AERO® 5100 Promoter (500)	+	152	147	155	151	4
6	AERO® 5100 Promoter (150)	+	140	139	136	138	2
7	AERO® 5100 Promoter (50)	+	103	81	87	90	11
8	Ethanol (0.1 ml)	+	106	104	92	101	8
9	AERO® 5100 Promoter (5000)	-	57 t	46 t	45 t	49	7
10	AERO® 5100 Promoter (1500)	-	60	73	66	66	7
11	AERO® 5100 Promoter (500)	-	89	87	74	83	8
12	AERO® 5100 Promoter (150)	-	92	84	94	90	5
13	AERO® 5100 Promoter (50)	-	66	87	103	85	19
14	Ethanol (0.1 ml)	-	107	97	117	107	10
15	Benzo[a]pyrene (5)	+	616	672	661	650	30
16	ENNG (3)	-	480	419	412	437	37
17	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	118	133	135	129	9
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

TABLE 3

Results obtained with *Salmonella typhimurium* TA1535 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 1				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	26 t	24 t	24 t	25	1
4	AERO® 5100 Promoter (1500)	+	189	240	203	211	26
5	AERO® 5100 Promoter (500)	+	130	119	125	125	6
6	AERO® 5100 Promoter (150)	+	79	79	71	76	5
7	AERO® 5100 Promoter (50)	+	35	32	39	35	4
8	AERO® 5100 Promoter (15)	+	26	23	26	25	2
9	AERO® 5100 Promoter (5)	+	14	19	16	16	3
10	Ethanol (0.1 ml)	+	16	19	14	16	3
11	AERO® 5100 Promoter (5000)	-	12 t	5 t	8 t	8	4
12	AERO® 5100 Promoter (1500)	-	14	13	13	13	1
13	AERO® 5100 Promoter (500)	-	17	19	13	16	3
14	AERO® 5100 Promoter (150)	-	15	13	13	14	1
15	AERO® 5100 Promoter (50)	-	17	13	13	14	2
16	AERO® 5100 Promoter (15)	-	15	13	17	15	2
17	AERO® 5100 Promoter (5)	-	14	13	17	15	2
18	Ethanol (0.1 ml)	-	14	22	20	19	4
19	2-Aminoanthracene (2)	+	329	407	406	381	45
20	ENNG (5)	-	130	155	158	148	15
21	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	109	111	104	108	4
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

TABLE 3 - continued

Results obtained with *Salmonella typhimurium* TA1535 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 2				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	13 t	16 t	13 t	14	2
4	AERO® 5100 Promoter (1500)	+	146	131	151	143	10
5	AERO® 5100 Promoter (500)	+	96	88	96	93	5
6	AERO® 5100 Promoter (150)	+	63	53	57	58	5
7	AERO® 5100 Promoter (50)	+	29	24	24	26	3
8	Ethanol (0.1 ml)	+	23	16	16	18	4
9	AERO® 5100 Promoter (5000)	-	5 t	6 t	5 t	5	1
10	AERO® 5100 Promoter (1500)	-	17	10	9	12	4
11	AERO® 5100 Promoter (500)	-	14	14	10	13	2
12	AERO® 5100 Promoter (150)	-	17	9	14	13	4
13	AERO® 5100 Promoter (50)	-	20	15	17	17	3
14	Ethanol (0.1 ml)	-	20	16	16	17	2
15	2-Aminoanthracene (2)	+	477	509	498	495	16
16	ENNG (5)	-	239	208	209	219	18
17	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	129	110	116	118	10
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

TABLE 4

Results obtained with *Salmonella typhimurium* TA1537 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 1				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	7 t	5 t	5 t	6	1
4	AERO® 5100 Promoter (1500)	+	14	9	8	10	3
5	AERO® 5100 Promoter (500)	+	9	10	9	9	1
6	AERO® 5100 Promoter (150)	+	10	12	9	10	2
7	AERO® 5100 Promoter (50)	+	10	9	9	9	1
8	AERO® 5100 Promoter (15)	+	10	12	12	11	1
9	AERO® 5100 Promoter (5)	+	14	12	15	14	2
10	Ethanol (0.1 ml)	+	15	13	13	14	1
11	AERO® 5100 Promoter (5000)	-	3 t	3 t	3 t	3	0
12	AERO® 5100 Promoter (1500)	-	13	12	9	11	2
13	AERO® 5100 Promoter (500)	-	13	13	9	12	2
14	AERO® 5100 Promoter (150)	-	10	12	10	11	1
15	AERO® 5100 Promoter (50)	-	10	13	12	12	2
16	AERO® 5100 Promoter (15)	-	12	13	10	12	2
17	AERO® 5100 Promoter (5)	-	14	10	7	10	4
18	Ethanol (0.1 ml)	-	15	14	10	13	3
19	Benzo[a]pyrene (5)	+	187	246	212	215	30
20	9-Aminoacridine (80)	-	785	1081	909	925	149
21	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	100	103	100	101	2
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

TABLE 4 - continued

Results obtained with *Salmonella typhimurium* TA1537 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 2				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	6 t	6 t	6 t	6	0
4	AERO® 5100 Promoter (1500)	+	9	9	13	10	2
5	AERO® 5100 Promoter (500)	+	8	12	9	10	2
6	AERO® 5100 Promoter (150)	+	7	8	6	7	1
7	AERO® 5100 Promoter (50)	+	10	6	6	7	2
8	Ethanol (0.1 ml)	+	12	13	14	13	1
9	AERO® 5100 Promoter (5000)	-	6 t	7 t	6 t	6	1
10	AERO® 5100 Promoter (1500)	-	13	9	10	11	2
11	AERO® 5100 Promoter (500)	-	13	10	13	12	2
12	AERO® 5100 Promoter (150)	-	10	10	14	11	2
13	AERO® 5100 Promoter (50)	-	13	15	13	14	1
14	Ethanol (0.1 ml)	-	15	14	13	14	1
15	Benzo[a]pyrene (5)	+	208	164	184	185	22
16	9-Aminoacridine (80)	-	1549	1156	1268	1324	202
17	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	111	110	109	110	1
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

TABLE 5

Results obtained with *Escherichia coli* CM891 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 1				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	43 t	52 t	65 t	53	11
4	AERO® 5100 Promoter (1500)	+	97	114	104	105	9
5	AERO® 5100 Promoter (500)	+	95	97	94	95	2
6	AERO® 5100 Promoter (150)	+	87	81	101	90	10
7	AERO® 5100 Promoter (50)	+	87	88	92	89	3
8	AERO® 5100 Promoter (15)	+	101	109	79	96	16
9	AERO® 5100 Promoter (5)	+	93	110	97	100	9
10	Ethanol (0.1 ml)	+	93	111	109	104	10
11	AERO® 5100 Promoter (5000)	-	78 t	50 t	55 t	61	15
12	AERO® 5100 Promoter (1500)	-	107	99	95	100	6
13	AERO® 5100 Promoter (500)	-	101	116	117	111	9
14	AERO® 5100 Promoter (150)	-	94	114	111	106	11
15	AERO® 5100 Promoter (50)	-	119	119	125	121	3
16	AERO® 5100 Promoter (15)	-	106	104	97	102	5
17	AERO® 5100 Promoter (5)	-	73	102	90	88	15
18	Ethanol (0.1 ml)	-	108	101	94	101	7
19	2-Aminoanthracene (10)	+	627	602	611	613	13
20	ENNG (2)	-	2332	2258	2347	2312	48
21	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	109	115	129	118	10
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

TABLE 5 - continued

Results obtained with *Escherichia coli* CM891 following exposure to AERO® 5100 Promoter

Plate No.	Addition (µg)	S9 mix + present - absent	Revertant colony counts and means				
			Test 2				
			A	B	C	Mean	sd
1	None; sterility check	+	0	0	0	0	0
2	AERO® 5100 Promoter (5000); sterility check	-	0	0	0	0	0
3	AERO® 5100 Promoter (5000)	+	84 t	67 t	66 t	72	10
4	AERO® 5100 Promoter (1500)	+	96	109	117	107	11
5	AERO® 5100 Promoter (500)	+	119	123	96	113	15
6	AERO® 5100 Promoter (150)	+	103	124	103	110	12
7	AERO® 5100 Promoter (50)	+	119	102	89	103	15
8	Ethanol (0.1 ml)	+	119	96	107	107	12
9	AERO® 5100 Promoter (5000)	-	41 t	29 t	41 t	37	7
10	AERO® 5100 Promoter (1500)	-	82	86	101	90	10
11	AERO® 5100 Promoter (500)	-	95	104	89	96	8
12	AERO® 5100 Promoter (150)	-	108	84	107	100	14
13	AERO® 5100 Promoter (50)	-	102	82	100	95	11
14	Ethanol (0.1 ml)	-	109	106	114	110	4
15	2-Aminoanthracene (10)	+	908	729	803	813	90
16	ENNG (2)	-	1759	1742	1859	1787	63
17	None; 10 ⁻⁶ dilution of overnight culture, plated on nutrient agar (total counts)	-	129	125	126	127	2
sd	Standard deviation						
t	Thinning of the background lawn of non-revertant cells was observed						

FIGURE 1

Dose response curves: test 1 (+ S9 mix)

Name of test substance: AERO® 5100 Promoter

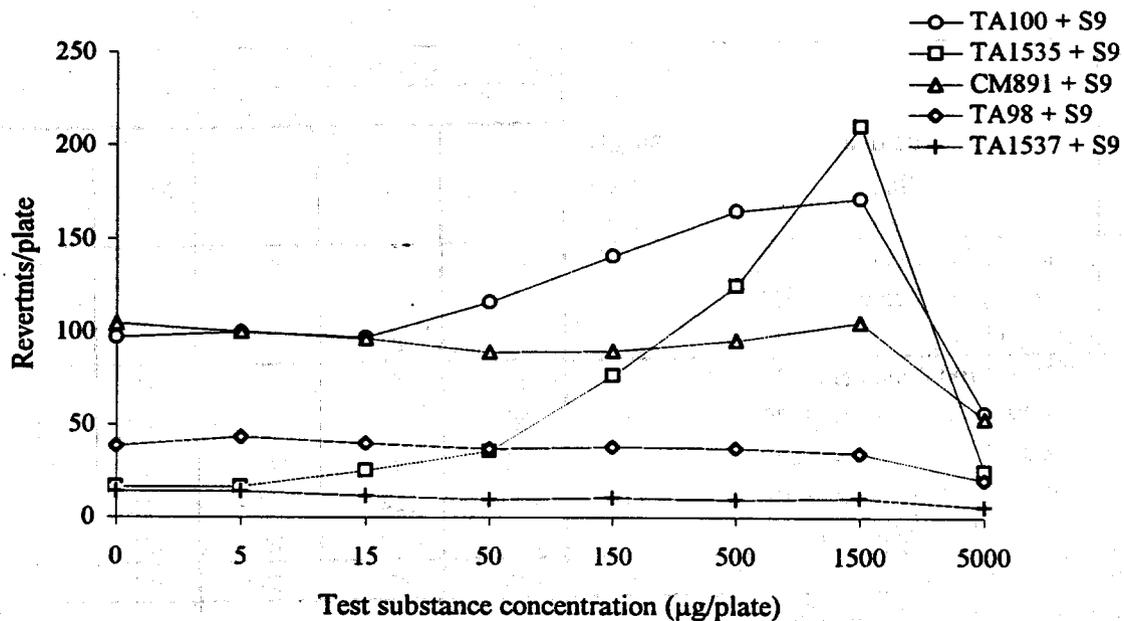


FIGURE 2

Dose response curves: test 1 (- S9 mix)

Name of test substance: AERO® 5100 Promoter

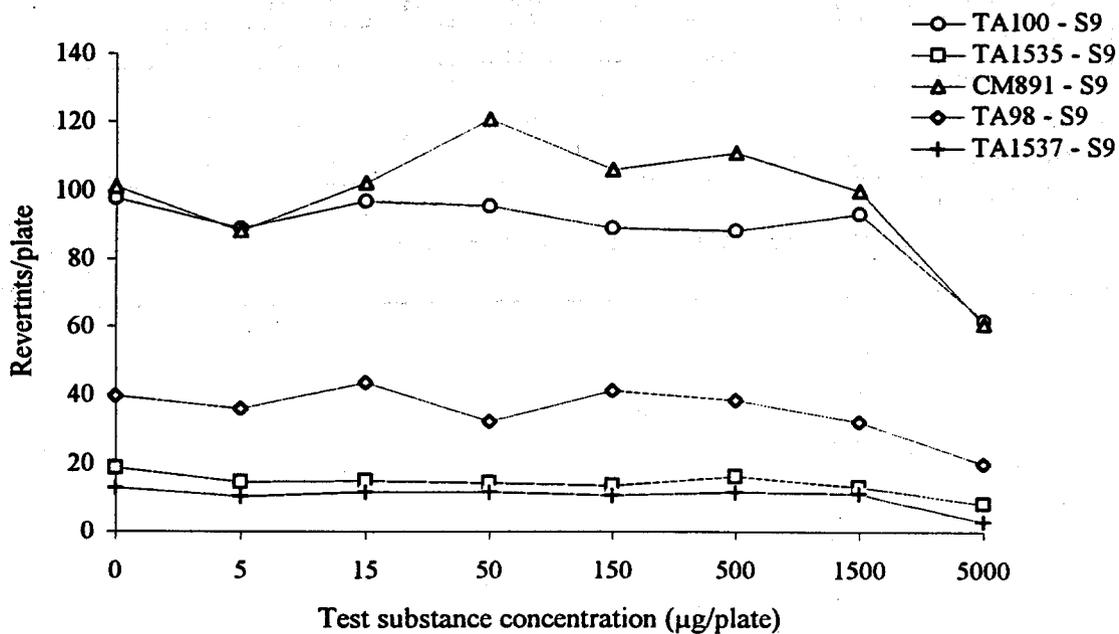


FIGURE 3

Dose response curves: test 2 (+ S9 mix)

Name of test substance: AERO® 5100 Promoter

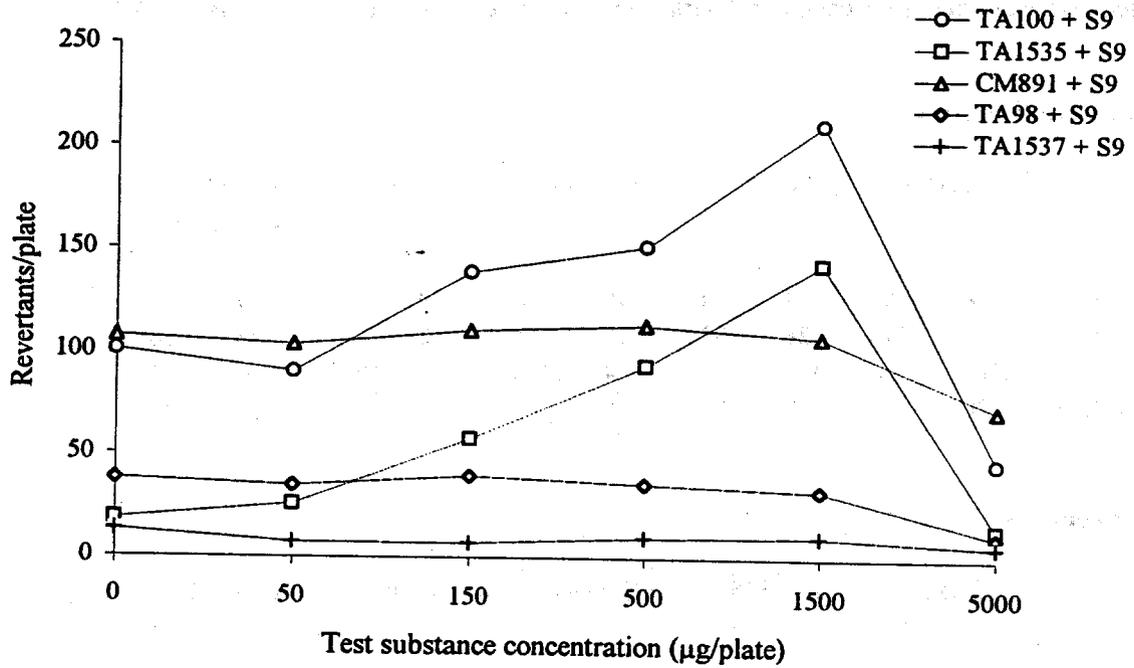
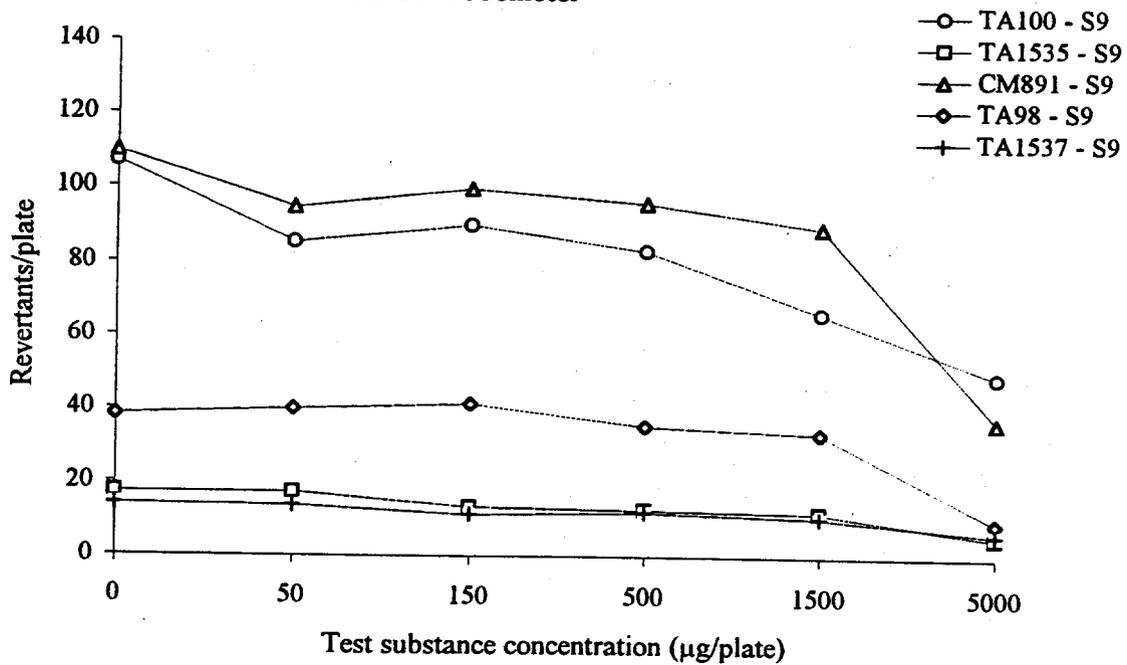


FIGURE 4

Dose response curves: test 2 (- S9 mix)

Name of test substance: AERO® 5100 Promoter



APPENDIX 1

Historical control data

Presented below are the historical control data from the period 1 April 1997 to 31 March 1998.

Ethanol solvent controls

Strain	TA100		TA1535		CM891		TA98		TA1537	
	-	+	-	+	-	+	-	+	-	+
Minimum	90	86	14	14	110	97	29	29	7	7
Maximum	121	118	28	33	182	179	42	43	18	18
Mean	98.6	99.0	19.2	20.8	120.4	120.0	36.6	37.6	12.4	12.6
No. of values	33	33	33	33	8	8	33	33	33	33

Positive controls

Strain	TA100		TA1535		CM891		TA98		TA1537	
	-	+	-	+	-	+	-	+	-	+
Minimum	190	240	37	80	294	231	117	200	562	101
Maximum	1477	1231	1243	457	2203	2097	649	765	4012	355
Mean	365.4	520.4	192.2	218.4	1223.9	664.7	246.1	472.5	1667.0	208.3
No. of values	225	224	219	218	76	76	222	221	219	218

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