RTC

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]7850
BACTERIAL MUTATION ASSAY
(*S. typhimurium* and *E. coli*)

FINAL REPORT

RTC Study no.: 52400

Sponsor:

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Total number of pages: 58

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C.C.I.A.A. n° 375376
Reg. Soc. Trib. di Roma n° 2828/72
Cod. Fisc.: 00653120584
Partita IVA: 00920611001

[redacted]7850
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(*S. typhimurium* and *E. coli*)

RTC Study no.: 52400

FINAL REPORT

I, the undersigned, was responsible for the preparation of this report.

[redacted]

[redacted]
(Study Director)

Date [redacted]

COMPLIANCE STATEMENT

We, the undersigned, hereby declare that the following report constitutes a true and faithful account of the procedures adopted, and the results obtained in the performance of the study. The aspects of the study conducted by Research Toxicology Centre S.p.A. were performed in accordance with:

A. *Good laboratory practice for non clinical laboratory studies*, U.S. Food and Drug Administration, Code of Federal Regulations, 21 Part 58, 22 December 1978 and subsequent revisions.

B. Decreto Legislativo 27 Gennaio 1992 n. 120, *Adoption of 88/320/EEC and 90/18/EEC Directives on the inspection and verification of good laboratory practice (G.U. 18 Febbraio 1992 n. 40)* and subsequent revisions.

C. Directive 2004/10/EC of European Parliament and of the Council of 11 February 2004. *On the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances.*

D. ENV/MC/CHEM(98)17 *OECD principles on Good Laboratory Practice (as revised in 1997).*

[redacted]

[redacted]
(Study Director)

Date [redacted]

[redacted]

[redacted]
(Scientific Director)

Date [redacted]

QUALITY ASSURANCE STATEMENT

(Relevant to those aspects of the study conducted by RTC S.p.A.)

Study phases monitored by RTC's QAU According to current relevant Standard Operating Procedures	Quality Assurance Inspections (Day Month Year)		
	Inspection	Report to Study Director	Report to Company Management
PROTOCOL CHECK	06.02.2006	07.02.2006	07.02.2006
PROCESS-BASED INSPECTIONS RELATED TO THIS TYPE OF STUDY			
Dose preparation	04.05.2006	-	11.05.2006
Treatment	17.01.2006	-	31.01.2006
Plating out	07.02.2006	-	27.03.2006
Plate scoring	03.03.2006	-	09.03.2006

Other process-based inspections were carried out on routine activities not directly related to this type of study. The relevant documentation is kept on file although specific inspection dates are not reported here.

Associated laboratories and support functions are subject to regular facility inspections.

FINAL REPORT Review of this report by RTC's QAU found the reported methods and procedures to describe those used and the results to constitute an accurate representation of the recorded raw data.	Review completed [redacted]

[redacted]

[redacted]

Date

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1. SUMMARY

1.1 The test item [redacted]7850 was examined for the ability to induce gene mutations in tester strains of *Salmonella typhimurium* and *Escherichia coli,* as measured by reversion of auxotrophic strains to prototrophy. The five tester strains TA1535, TA1537, TA98, TA100 and WP2 *uvr*A were used. Experiments were performed both in the absence and presence of metabolic activation, using liver S9 fraction from rats pre-treated with phenobarbitone and betanaphthoflavone.
Test item solutions were prepared using ethanol.

1.2 In the toxicity test, the test item was assayed at a maximum dose-level of 5000 µg/plate and at four lower dose-levels: 1580, 500, 158 and 50.0 µg/plate. In the absence of S9 metabolic activation, signs of toxicity were observed at higher dose-levels with TA1535, TA1537 and WP2*uvr*A tester strains and at the highest dose-level with TA98 and TA100. In the presence of S9 metabolism, signs of toxicity were observed at the highest concentration tested with TA1535 and TA100.

Two main experiments were performed. In Main Assay I, using the plate incorporation method, treatments were performed using the following dose-levels:

Tester strain	S9	Dose-levels (µg/plate)
TA1535	–	200, 100, 50.0, 25.0, 12.5, 6.25
TA1537	–	100, 50.0, 25.0, 12.5, 6.25, 3.13
WP2*uvr*A	–	50.0, 25.0, 12.5, 6.25, 3.13, 1.56
TA98, TA100	–	5000, 2500, 1250, 625, 313, 156
TA1535, TA100	+	
TA1537, TA98, WP2*uvr*A	+	5000, 2500, 1250, 625, 313

As no increases in revertant numbers were observed, all treatments of Main Assay II included a pre-incubation step. Toxicity results obtained in the first experiment were taken into consideration selecting the dose-levels for Main Assay II. Concentrations tested were as follows:

Tester strains	S9	Dose levels (µg/plate)
TA1535	–	200, 100, 50.0, 25.0, 12.5, 6.25
TA1537	–	100, 50.0, 25.0, 12.5, 6.25, 3.13
WP2*uvr*A	–	50.0, 25.0, 12.5, 6.25, 3.13, 1.56
TA98, TA100	–	2500, 1250, 625, 313, 156, 78.1
TA1535, TA1537	+	5000, 2500, 1250, 625, 313, 156, 78.1
TA98, TA100, WP2*uvr*A	+	5000, 2500, 1250, 625, 313, 156

No toxcity was observed in the absence of S9 metabolism with WP2*uvr*A tester strain using the plate inorporation or the pre-incubation method. In order to evaluate a potential mutagenic effect up to cytotoxic concentrations, an additional experiment was carried out with this tester strain using a maximum concentration of 800 µg/plate and four lower dose-levels. Treatments were performed using both the plate incorporation and the pre-incubation method.

1.3 The test item did not induce two fold increases in the number of revertant colonies in the plate incorporation or pre-incubation assays, at any dose-level, in any tester strain, in the absence or presence of S9 metabolism.

1.4 It is concluded that the test item [redacted] 7850 does not induce reverse mutation in *Salmonella typhimurium* or *Escherichia coli* under the reported experimental conditions.

2. INTRODUCTION

2.1 Purpose

This report describes experiments performed to assess the mutagenic activity of the test item to *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100, and to *Escherichia coli* strain WP2 *uvr*A using the procedures developed by Ames *et al.*, 1975 and revised by Maron and Ames, 1983.

The study was designed to comply with the experimental methods indicated in:
- EEC Council Directive 2000/32, Annex 4D.
- OECD Guideline for the testing of chemicals No. 471 (Adopted July 1997).
- ICH S2A Genotoxicity: Specific Aspects of Regulatory Tests, Step 5.

2.2 Principles of the method

Reverse mutation assays employ bacterial strains which are already mutant at a locus whose phenotypic effects are easily detected. The *Salmonella* tester strains have mutations causing dependence on a particular amino acid (histidine) for growth. The ability of test items to cause reverse mutations (reversions) to histidine-independence can easily be measured. The *E. coli* tester strains of the WP2 series are similarly mutant at the tryptophan locus.

Since many chemicals only demonstrate mutagenic activity after metabolism to reactive forms, in order to detect these "indirect mutagens" the test is performed in the presence and absence of a rat liver metabolising system.

2.3 Study organisation

Sponsor:

SOLVAY SOLEXIS S.p.A.
Viale Lombardia, 20
20021 Bollate (MI)
Italy

Location of Study:

Research Toxicology Centre S.p.A.
Genetic Toxicology Department
Via Tito Speri, 12
00040 Pomezia (Rome)
Italy

Principal dates:

Study protocol approved by Study Director: 18-Jan-2006
Study commenced: 28-Feb-2006 (Toxicity assay treatment)
Study completed: 17-Mar-2006 (Completion of scoring Main Assay III)

Study Director:

[redacted]

Archiving:

The original data arising from this study and a copy of the final report consigned will be stored in the archives of Research Toxicology Centre S.p.A. for a period of 3 years from the date of consignment of the report. At the completion of this period the Sponsor will be contacted for despatch or disposal of the material, or further archiving. An aliquot of the test item will be retained within the archives of the testing facility for a period of 10 years after which it will be destroyed.

3. MATERIALS AND METHODS

3.1 Test item

Details of the test item received at RTC were as follows:

Name	:	[redacted] 7850
Batch	:	3223 ON
Purity	:	>75% (referred to carboxylic end groups)
Received from	:	Solvay Solexis
Date received	:	20-Feb-2006
Amount received	:	100 g
Description	:	colourless liquid
Expiry date	:	2012
Container	:	opaque plastic bottle
Storage at RTC	:	room temperature
RTC reference number	:	10022

On 27-Feb-2006 a 12 g sub-sample of the test item was transferred from the Formulation Unit to the Department of Genetic and Cellular Toxicology and stored under the same conditions.

Solutions of the test item, as received, were prepared immediately before use in ethanol. Solutions were prepared on a weight/volume basis without correction for the displacement due to the volume of the test item. Concentrations were expressed in terms of material as received. All test item solutions were used within 2 hours and 30 minutes of the initial formulation. No assay of test item stability, nor its concentration and homogeneity in solvent were undertaken. All dose-levels in this report are expressed to three significant figures.

3.2 Control items

The solvents used in this study were:
Sterile distilled water (Bieffe Medital, batch 03H2802).
Dimethylsulphoxide (DMSO) (Fluka AG, batch 1060564 41204083).
Ethanol (Carlo Erba, batch 4G297304I)

Positive control treatments used solutions prepared as follows:

Sodium azide (Moltox, Inc., batch 6258SA) in distilled water.
9-Aminoacridine (Moltox, Inc., batch 6030AC) in DMSO.
2-Nitrofluorene (Moltox, Inc., batch 2188NF) in DMSO.
2-Aminoanthracene (Moltox, Inc., batch 6269AA and Sigma, batch 58F-3462) in DMSO.
Methylmethanesulphonate (MMS) (Fluka AG, batch 359316/153696) in distilled water.

3.3 Media

The following growth media were used:

Nutrient Broth: Oxoid Nutrient Broth No 2 was prepared at a concentration of 2.5% in distilled water and autoclaved prior to use.
This was used for the preparation of liquid cultures of the tester strains.

Nutrient Agar: Oxoid Nutrient Broth No 2 (25g) and Difco Bacto-agar (15g) were added to distilled water (1 litre) and autoclaved.
The solutions were then poured into 9 cm plastic Petri dishes and allowed to solidify and dry before use. These plates were used for the non-selective growth of the tester strains.

Minimal Agar: Minimal medium agar was prepared as 1.5% Difco Bacto-agar in Vogel-Bonner Medium E, with 2% Glucose, and poured into 9 cm plastic Petri dishes.

Top Agar: "Top Agar" (overlay agar) was prepared as 0.6% Difco Bacto-agar + 0.5% NaCl in distilled water. Prior to use 10 ml of a sterile solution of 0.5 mM Biotin + 0.5 mM Histidine (or 0.5 mM tryptophan) were added to the top agar (100 ml).

3.4 S9 tissue homogenate

One batch of S9 tissue homogenate (designated 2006/1) was used in this study and had the following characteristics:

S9 Batch	Protein content (mg/ml)	Aminopyrine demethylase activity (µM/g liver/5 min, formaldehyde production)
2006/1	36.5 ± 2.42	4.59 ± 0.15

The S9 tissue fraction was prepared from the livers of five young male Sprague-Dawley rats which had received prior treatment with phenobarbital and betanaphthoflavone to induce high levels of xenobiotic metabolising enzymes. The efficacy of the S9 tissue fraction was previously checked in an Ames test and produced acceptable responses with the indirect mutagens 2-aminoanthracene and benzo(a)pyrene, using *S. typhimurium* tester strain TA100.

	Induced *Salmonella typhimurium* revertants:	
TA100	2-Aminoanthracene (1 µg/plate)	1557 ± 197
	Benzo(a)pyrene (2.5 µg/plate)	514 ± 27

The mixture of S9 tissue fraction and cofactors (S9 mix) was prepared as follows (for each 10 ml):

S9 tissue fraction	1.0 ml
NADP (100 mM)	0.4 ml
G-6-P (100 mM)	0.5 ml
KCl (330 mM)	1.0 ml
MgCl2 (100 mM)	0.8 ml
Phosphate buffer (pH 7.4, 200 mM)	5.0 ml
Distilled Water	1.3 ml
	10.0 ml

3.5 Bacterial strains

Four strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and a strain of *Escherichia coli* (WP2 *uvr*A) were used in this study. Permanent stocks of these strains are kept at -80°C in RTC. Overnight subcultures of these stocks were prepared for each day's work.
Bacteria were taken from vials of frozen cultures, which had been checked for the presence of the appropriate genetic markers, as follows:

Histidine requirement	:	No Growth on Minimal plates + Biotin. Growth on Minimal plates + Biotin + Histidine.
Tryptophan requirement	:	No Growth on Minimal agar plates Growth on Minimal plates + Tryptophan.
*uvr*A, *uvr*B	:	Sensitivity to UV irradiation.
rfa	:	Sensitivity to Crystal Violet.
pKM101	:	Resistance to Ampicillin.

Bacterial cultures in liquid and on agar were clearly identified with their identity.

3.6 Methods

3.6.1 Preliminary toxicity test

A preliminary toxicity test was undertaken in order to select the concentrations of the test item to be used in the main assays. In this test a wide range of dose-levels of the test item, set at half-log intervals, was used. Treatments were performed both in the absence and presence of S9 metabolism using the plate incorporation method; a single plate was used at each test point and positive controls were not included. Toxicity was assessed on the basis of a decline in the number of spontaneous revertants, a thinning of the background lawn or a microcolony formation.

3.6.2 Main experiments

Three experiments were performed including negative and positive controls in the absence and presence of an S9 metabolising system. Three replicate plates were used at each test point.

In addition, plates were prepared to check the sterility of the test item solutions and the S9 mix, and dilutions of the bacterial cultures were plated on nutrient agar plates to establish the number of bacteria in the cultures.

The first experiment and a treatment series of the third experiment were performed using the plate-incorporation method. The components of the assay (the tester strain bacteria, the test item and S9 mix or phosphate buffer) were added to molten overlay agar and vortexed. The mixture was then poured onto the surface of a minimal medium agar plate, and allowed to solidify prior to incubation.

The overlay mixture was composed as follows:

(i)	Overlay agar (held at 45°C)	2 ml
(ii)	Test or control item solution	0.1 ml
(iii)	S9 mix or phosphate buffer (pH 7.4, 0.1 M)	0.5 ml
(iv)	Bacterial suspension	0.1 ml

The second experiment and a treatment series of the third experiment were performed using the pre-incubation method.
The components were added in turn to an empty test-tube:

(i)	Bacterial suspension	0.1 ml
(ii)	Test item solution	0.01 ml
	or DMSO or positive control solution	0.05 ml
(iii)	S9 mix or phosphate buffer (pH 7.4, 0.1 M)	0.5 ml

The incubate was vortexed and placed at 37°C for 30 minutes. Two ml of overlay agar were then added and the mixture vortexed again and poured onto the surface of a minimal medium agar plate and allowed to solidify.

3.6.3 Incubation and scoring

The prepared plates were inverted and incubated for approximately 72 hours at 37°C. After this period of incubation, the scoring was effected by counting the number of revertant colonies on each plate.

4. RESULTS

4.1 Solubility test

As indicated by the Sponsor, the test item was found to be soluble in ethanol at a concentration of 500 mg/ml. This solvent was selected since it is compatible with the survival of the bacteria and the S9 metabolic activity.
Since 100 µl of the test item solution are used in the preparation of each plate, this permitted a maximum concentration of 5000 µg/plate to be used in the toxicity test (the upper limit to testing indicated by the Study Protocol).

4.2 Toxicity test

The test item was assayed using the plate incorporation method, at a maximum dose-level of 5000 µg/plate and at four lower dose-levels spaced at approximately half-log intervals: 1580, 500, 158 and 50.0 µg/plate. Results are presented in Tables 1 and 2.

In the absence of S9 metabolic activation, toxicity, as indicated by lack of colony growth, thinning of the background lawn and reduction in revertant numbers, was observed at higher dose-levels with TA1535, TA1537 and WP2*uvr*A and at the highest dose-level with TA98 and TA100 tester strains. In the presence of S9 metabolism, toxicity was observed only at the highest concentration tested with TA1535 and TA100 tester strains.

4.3 Assay for reverse mutation

Two experiments were performed; individual plate counts for these tests, and the mean and standard error of the mean for each test point, together with statistical analysis are presented in Tables 3 to 20.

In Main Assay I, using the plate incorporation method, the test items were assayed at the following dose-levels:

Tester strain	S9	Dose-levels (µg/plate)
TA1535	–	200, 100, 50.0, 25.0, 12.5, 6.25
TA1537	–	100, 50.0, 25.0, 12.5, 6.25, 3.13
WP2*uvr*A	–	50.0, 25.0, 12.5, 6.25, 3.13, 1.56
TA98, TA100	–	5000, 2500, 1250, 625, 313, 156
TA1535, TA100	+	
TA1537, TA98, WP2*uvr*A	+	5000, 2500, 1250, 625, 313

Following treatment with [redacted] 7850 in the absence of S9 metabolic activation, slight toxicity, as indicated by thinning of the background lawn, was observed at the highest concentration tested with TA1535 and TA1537 tester strains. With TA 98 and TA100 tester strains, reduction in revertant numbers was observed at 2500 and 5000 µg/plate, while no relevant toxicity was observed with WP2*uvr*A.

Treatments in the presence of S9 metabolic activation showed thinning of the background lawn and/or reduction in revertant numbers at the highest concentration tested (5000 µg/plate).

As no increases in revertant numbers were observed, all treatments of Main Assay II included a pre-incubation step. Toxicity results obtained in the first experiment were taken into consideration selecting dose-levels for Main Assay II. Concentrations tested were as follow:

Tester strains	S9	Dose levels (µg/plate)
TA1535	–	200, 100, 50.0, 25.0, 12.5, 6.25
TA1537	–	100, 50.0, 25.0, 12.5, 6.25, 3.13
WP2*uvr*A	–	50.0, 25.0, 12.5, 6.25, 3.13, 1.56
TA98, TA100	–	2500, 1250, 625, 313, 156, 78.1
TA1535, TA1537	+	5000, 2500, 1250, 625, 313, 156, 78.1
TA98, TA100, WP2*uvr*A	+	5000, 2500, 1250, 625, 313, 156

In the absence of S9 metabolism, toxicity as indicated by thinning of the background lawn and reduction in revertant numbers was observed at the highest dose level tested with TA1535, TA1537 and TA98 tester strains. Signs of toxicity were also observed with TA100 over a concentration range from 625 to 2500 µg/plate. In the presence of S9 metabolism toxicity, as indicated by thinning of the background lawn and reduction in revertant colonies, was observed at the three highest dose levels with all tester strains.

No sign of toxicity was observed in the absence of S9 metabolism with WP2*uvr*A tester strain using the plate inorporation or the pre-incubation method. In order to evaluate a potential mutagenic effect up to cytotoxic concentrations, an additional experiment was carried out with this tester strain using a maximum concentration of 800 µg/plate and four lower dose-levels spaced by a factor of two. Treatments were performed using both the plate incorporation and the pre-incubation method.

No visible precipitate was observed in any experiment, at the end of the incubation period even at the highest concentration tested.

No treatments with the test item produced increases in revertant numbers in the plate incorporation or pre-incubation assay, in any tester strain, in the absence or presence of S9 metabolism.

The sterility of the S9 mix and the test item solutions was confirmed by the absence of colonies on additional agar plates spread separately with these solutions. In Main Assay III, sterility data of test item solutions, used for plate incorporation treatment, were not recorded. However, no contamination was observed in any plate treated with the test item at any dose level. Hence, this deviation to the Study Protocol was not considered to have affected the integrity of the study. Marked increases in revertant numbers were obtained in these tests following treatment with the positive control items, indicating that the assay system was functioning correctly.

5. ANALYSIS OF RESULTS

5.1 Criteria for outcome of the assays

For the test item to be considered mutagenic, two-fold (or more) increases in mean revertant numbers must be observed at two consecutive dose-levels or at the highest practicable dose-level only. In addition, there must be evidence of a dose-response relationship showing increasing numbers of mutant colonies with increasing dose-levels.

5.2 Evaluation

The test item does not induce two-fold increases in the number of revertant colonies, at any dose-level, in any tester strain, in the absence or presence of S9 metabolism. On the basis of the stated criteria it must be concluded that the test item [redacted] 7850 is not mutagenic to *S. typhimurium* and *E. coli* under the reported experimental conditions.

6. CONCLUSION

It is concluded that the test item [redacted]7850 does not induce reverse mutation in *Salmonella typhimurium* or *Escherichia coli* under the reported experimental conditions.

7. KEY TO TABLES 1-20

7.1 Structure of Tables 3-20

These tables show, for each *Salmonella typhimurium* or *Escherichia coli* tester strain, the individual plate counts obtained for the negative and positive controls, and at each dose-level of the test item. The mean number of revertant colonies and standard error of the mean are also presented. The "untreated" plates receive no treatment, while the 0.00 dose-level is the solvent control. The titre of the bacterial cultures is given (million cells/plate).

7.2 Regression line

i) The regression analysis fits a regression line to the data by the least squares method, after square root transformation of the plate counts to satisfy normal distribution and homoscedasticity assumptions. The regression equation is expressed as:

$y = a + bx$

where y = transformed revertant numbers
a = intercept
b = slope value
x = dose-level (in the units given).

ii) The regression line does not include the untreated control data, but includes the solvent control data.

iii) Regression lines are calculated using a minimum of the three lowest dose-levels, and then including the further dose-levels in turn. The correlation co-efficient (r), the value of students "t" statistic, and the p-value for the regression lines are also given.

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 1 - Toxicity test without metabolic activation

STUDY NO.: 52400

SOLVENT: Ethanol

EXPERIMENT: Toxicity test

Dose-level (µg/plate)	TA-1535 Rev/pl.	TA-1537 Rev/pl.	TA-98 Rev/pl.	TA-100 Rev/pl.	WP2 *uvr*A Rev/pl.
Untreated	16	21	28	120	25
0.00	19	17	33	118	30
50.0	15	19	36	123	18
158	9	0 *	32	134	15
500	10	0 *	39	126	21
1580	9	0 *	38	111	21
5000	6	0 *	16	70	19

*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 2 - Toxicity test with metabolic activation

STUDY NO.: 52400

SOLVENT: Ethanol

EXPERIMENT: Toxicity test

Dose-level (µg/plate)	TA-1535 Rev/pl.	TA-1537 Rev/pl.	TA-98 Rev/pl.	TA-100 Rev/pl.	WP2 *uvr*A Rev/pl.
Untreated	17	24	44	132	31
0.00	21	19	46	110	28
50.0	17	25	34	116	32
158	15	24	43	118	32
500	12	24	47	117	34
1580	16	19	46	120	28
5000	8 *	19	37	94	29

*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 3 - Experiment I - Plate incorporation method - TA1535

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA1535 Titre: 222

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.
Untreated	18	17	14	16	1.2
0.00	15	18	20	18	1.5
6.25	14	13	10	12	1.2
12.5	10	13	16	13	1.7
25.0	15	15	11	14	1.3
50.0	13	11	16	13	1.5
100	16	8	14	13	2.4
200	10 *	10 *	13 *	11	1.0

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.066	-0.0485	-0.59240	1.9455	0.09278
1 - 4	-	3.899	-0.0142	-0.33941	1.1410	0.28045
1 - 5	-	3.820	-0.0052	-0.24698	0.9190	0.37485
1 - 6	-	3.789	-0.0031	-0.26109	1.0819	0.29533
1 - 7	-	3.773	-0.0024	-0.40482	1.9298	0.06870

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	18	17	14	16	1.2
Sodium Azide	1 µg/pl	-	478	470	550	499	25.4

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*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 4 - Experiment I - Plate incorporation method - TA1535

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA1535 Titre: 222

Dose-level [µg/pl]	With metabolic activation Plate counts			Mean	S. E.
Untreated	16	13	14	14	0.9
0.00	19	15	19	18	1.3
156	12	17	16	15	1.5
313	14	18	17	16	1.2
625	18	12	16	15	1.8
1250	10	10	15	12	1.7
2500	12	9	14	12	1.5
5000	7 *	5 *	7 *	6	0.7

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	+	4.112	-0.0005	-0.23377	0.6361	0.54493
1 - 4	+	4.090	-0.0003	-0.25670	0.8399	0.42060
1 - 5	+	4.138	-0.0006	-0.62840	2.9127	0.01211
1 - 6	+	4.046	-0.0003	-0.62943	3.2400	0.00513
1 - 7	+	4.047	-0.0003	-0.85711	7.2527	0.00000

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µl/pl	+	17	17	20	18	1.0
2-Aminoanthracene	1 µg/pl	+	141	131	156	143	7.3

*: Tinning of the background lawn

[redacted]7850:
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TABLE 5 - Experiment I - Plate incorporation method - TA1537

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA1537 Titre: 220

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.
Untreated	16	16	19	17	1.0
0.00	12	12	11	12	0.3
3.13	13	14	12	13	0.6
6.25	13	13	15	14	0.7
12.5	13	12	15	13	0.9
25.0	11	11	12	11	0.3
50.0	11	12	7	10	1.5
100	14 *	14 *	13 *	14	0.3

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	3.431	0.0448	0.72587	2.7921	0.02683
1 - 4	-	3.500	0.0165	0.46596	1.6653	0.12682
1 - 5	-	3.596	-0.0054	-0.27144	1.0168	0.32778
1 - 6	-	3.622	-0.0089	-0.58170	2.8606	0.01133
1 - 7	-	3.510	0.0000	-0.00110	0.0048	0.99622

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µl/pl	-	11	14	13	13	0.9
9-Aminoacridine	50 µg/pl	-	168	141	201	170	17.3

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*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 6 - Experiment I - Plate incorporation method - TA1537

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA1537 Titre: 220

Dose-level [µg/pl]	With metabolic activation Plate counts			Mean	S. E.
Untreated	16	18	19	18	0.9
0.00	10	14	15	13	1.5
313	13	12	17	14	1.5
625	13	9	16	13	2.0
1250	12	12	11	12	0.3
2500	10	13	8	10	1.5
5000	7	7	6	7	0.3

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	+	3.648	-0.0001	-0.06665	0.1767	0.86472
1 - 4	+	3.672	-0.0002	-0.27600	0.9080	0.38521
1 - 5	+	3.672	-0.0002	-0.47809	1.9626	0.07146
1 - 6	+	3.693	-0.0002	-0.79504	5.2430	0.00008

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µl/pl	+	16	15	18	16	0.9
2-Aminoanthracene	1 µg/pl	+	106	92	104	101	4.4

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 7 - Experiment I - Plate incorporation method - WP2*uvr*A

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: WP2*uvr*A Titre: 300

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.
Untreated	26	28	24	26	1.2
0.00	24	22	21	22	0.9
1.56	20	27	22	23	2.1
3.13	25	28	27	27	0.9
6.25	25	27	23	25	1.2
12.5	20	26	24	23	1.8
25.0	19	26	27	24	2.5
50.0	14	18	23	18	2.6

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.672	0.1402	0.63811	2.1927	0.06442
1 - 4	-	4.782	0.0496	0.44022	1.5504	0.15208
1 - 5	-	4.883	0.0033	0.05588	0.2018	0.84319
1 - 6	-	4.895	0.0002	0.00602	0.0241	0.98108
1 - 7	-	4.970	-0.0117	-0.51111	2.5920	0.01789

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	26	28	24	26	1.2
MMS	500 µg/pl	-	142	153	165	153	6.6

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 8 - Experiment I - Plate incorporation method - WP2*uvr*A

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: WP2*uvr*A Titre: 300

Dose-level [µg/pl]	With metabolic activation Plate counts			Mean	S. E.
Untreated	31	34	30	32	1.2
0.00	30	31	28	30	0.9
313	42	33	30	35	3.6
625	32	40	34	35	2.4
1250	26	25	31	27	1.9
2500	30	31	29	30	0.6
5000	33 *	30 *	27 *	30	1.7

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	+	5.515	0.0008	0.53479	1.6745	0.13795
1 - 4	+	5.771	-0.0003	-0.29055	0.9602	0.35959
1 - 5	+	5.707	-0.0001	-0.27263	1.0217	0.32556
1 - 6	+	5.656	0.0000	-0.23537	0.9687	0.34712

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µl/pl	+	29	23	31	28	2.4
2-Aminoanthracene	10 µg/pl	+	179	146	189	171	13.0

*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 9 - Experiment I - Plate incorporation method - TA98

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA98 Titre: 251

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	34	32	27	31	2.1	34	35	44	38	3.2
0.00	39	37	34	37	1.5	43	49	47	46	1.8
156	34	36	45	38	3.4	NT	NT	NT		
313	34	34	40	36	2.0	45	44	38	42	2.2
625	30	36	40	35	2.9	48	46	43	46	1.5
1250	30	28	24	27	1.8	36	34	27	32	2.7
2500	9	8	18	12	3.2	40	42	42	41	0.7
5000	10	11	15	12	1.5	26 *	28 *	21 *	25	2.1

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	6.105	-0.0002	-0.08244	0.2189	0.83301
1 - 4	-	6.116	-0.0003	-0.20636	0.6669	0.51991
1 - 5	-	6.212	-0.0007	-0.71504	3.6879	0.00273
1 - 6	-	6.365	-0.0011	-0.91505	9.0745	0.00000
1 - 7	-	6.049	-0.0006	-0.86324	7.4542	0.00000
1 - 3	+	6.712	-0.0001	-0.08399	0.2230	0.82990
1 - 4	+	6.900	-0.0009	-0.76511	3.7576	0.00374
1 - 5	+	6.610	-0.0002	-0.36138	1.3974	0.18568
1 - 6	+	6.707	-0.0003	-0.78730	5.1076	0.00011

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µl/pl	-	30	27	31	29	1.2
2-Nitrofluorene	2 µg/pl	-	138	184	180	167	14.7
DMSO	100 µl/pl	+	37	40	35	37	1.5
2-Aminoanthracene	1 µg/pl	+	523	542	550	538	8.0

* : Thinning of the background lawn
NT: Not tested

[redacted]7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 10 - Experiment I - Plate incorporation method - TA100

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA100 Titre: 277

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	140	119	137	132	6.6	140	126	114	127	7.5
0.00	112	135	111	119	7.8	130	125	117	124	3.8
156	111	101	108	107	3.0	126	110	132	123	6.6
313	104	101	98	101	1.7	130	112	102	115	8.2
625	90	101	110	100	5.8	141	127	119	129	6.4
1250	104	99	96	100	2.3	117	102	121	113	5.8
2500	87	86	78	84	2.8	111	119	124	118	3.8
5000	83	61	54	66	8.7	78	96	88	87	5.2

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	10.860	-0.0028	-0.73205	2.8430	0.02494
1 - 4	-	10.686	-0.0013	-0.61350	2.4567	0.03387
1 - 5	-	10.520	-0.0006	-0.52691	2.2353	0.04358
1 - 6	-	10.515	-0.0006	-0.78101	5.0024	0.00013
1 - 7	-	10.477	-0.0005	-0.87495	7.8762	0.00000
1 - 3	+	11.184	-0.0014	-0.38812	1.1142	0.30197
1 - 4	+	10.976	0.0003	0.15202	0.4864	0.63718
1 - 5	+	11.103	-0.0003	-0.24294	0.9030	0.38296
1 - 6	+	11.042	-0.0001	-0.19440	0.7927	0.43953
1 - 7	+	11.180	-0.0003	-0.74201	4.8246	0.00012

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	140	119	137	132	6.6
Sodium Azide	1 µg/pl	-	681	796	722	733	33.7
DMSO	100 µl/pl	+	118	119	124	120	1.9
2-Aminoanthracene	1 µg/pl	+	1011	1449	1584	1348	172.9

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 11 - Experiment II - Pre-incubation method - TA1535

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA1535 Titre: 234

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.
Untreated	16	17	14	16	0.9
0.00	18	17	13	16	1.5
6.25	17	14	17	16	1.0
12.5	17	17	18	17	0.3
25.0	16	23	19	19	2.0
50.0	16	13	18	16	1.5
100	8	13	8	10	1.7
200	11 *	11 *	19 *	14	2.7

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	3.964	0.0138	0.33682	0.9464	0.37545
1 - 4	-	3.948	0.0169	0.55260	2.0967	0.06242
1 - 5	-	4.096	0.0000	0.00140	0.0051	0.99604
1 - 6	-	4.241	-0.0097	-0.68671	3.7787	0.00165
1 - 7	-	4.083	-0.0034	-0.46161	2.2682	0.03516

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	16	17	14	16	0.9
Sodium Azide	1 µg/pl	-	397	571	564	511	56.9

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*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 12 - Experiment II - Pre-incubation method - TA1535

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA1535 Titre: 234

Dose-level [µg/pl]	With metabolic activation Plate counts			Mean	S. E.
Untreated	16	12	17	15	1.5
0.00	15	19	20	18	1.5
78.1	19	17	11	16	2.4
156	19	19	25	21	2.0
313	15	16	14	15	0.6
625	15	16	21	17	1.9
1250	15 *	12 *	17 *	15	1.5
2500	14 *	13 *	20 *	16	2.2
5000	9 *	14 *	19 *	14	2.9

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	+	4.078	0.0022	0.31812	0.8878	0.40413
1 - 4	+	4.255	-0.0007	-0.20852	0.6742	0.51545
1 - 5	+	4.191	-0.0002	-0.09241	0.3346	0.74326
1 - 6	+	4.215	-0.0003	-0.31569	1.3308	0.20190
1 - 7	+	4.162	-0.0001	-0.26043	1.1757	0.25422
1 - 8	+	4.147	-0.0001	-0.35396	1.7751	0.08972

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	50 µl/pl	+	16	15	17	16	0.6
2-Aminoanthracene	1 µg/pl	+	99	103	82	95	6.4

*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 13 - Experiment II - Pre-incubation method - TA1537

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA1537 Titre: 318

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.
Untreated	19	22	17	19	1.5
0.00	16	12	19	16	2.0
3.13	14	19	19	17	1.7
6.25	14	16	15	15	0.6
12.5	21	15	17	18	1.8
25.0	15	19	14	16	1.5
50.0	21	15	18	18	1.7
100	13 *	16 *	14 *	14	0.9

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.023	-0.0111	-0.09346	0.2484	0.81098
1 - 4	-	3.962	0.0143	0.21094	0.6824	0.51048
1 - 5	-	4.019	0.0012	0.03439	0.1241	0.90316
1 - 6	-	3.997	0.0041	0.22852	0.9389	0.36173
1 - 7	-	4.070	-0.0017	-0.17753	0.7863	0.44136

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	50 µl/pl	-	21	19	15	18	1.8
9-Aminoacridine	50 µg/pl	-	140	153	181	158	12.1

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*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 14 - Experiment II - Pre-incubation method - TA1537

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA1537 Titre: 318

Dose-level [µg/pl]	With metabolic activation Plate counts			Mean	S. E.
Untreated	19	20	23	21	1.2
0.00	22	17	19	19	1.5
78.1	18	18	26	21	2.7
156	16	19	16	17	1.0
313	17	19	19	18	0.7
625	16	11	13	13	1.5
1250	7 *	6 *	4 *	6	0.9
2500	10 *	3 *	5 *	6	2.1
5000	5 *	4 *	8 *	6	1.2

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	+	4.482	-0.0017	-0.33243	0.9326	0.38209
1 - 4	+	4.413	-0.0006	-0.23988	0.7814	0.45268
1 - 5	+	4.482	-0.0012	-0.68029	3.3465	0.00526
1 - 6	+	4.563	-0.0017	-0.91939	9.3491	0.00000
1 - 7	+	4.330	-0.0009	-0.84629	6.9246	0.00000
1 - 8	+	4.068	-0.0005	-0.75229	5.3557	0.00002

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	50 µl/pl	+	20	22	23	22	0.9
2-Aminoanthracene	1 µg/pl	+	74	98	95	89	7.5

*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 15 - Experiment II - Pre-incubation method - WP2*uvr*A

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: WP2*uvr*A Titre: 288

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.
Untreated	30	18	25	24	3.5
0.00	20	26	25	24	1.9
1.56	26	18	27	24	2.8
3.13	27	33	32	31	1.9
6.25	19	30	34	28	4.5
12.5	22	24	30	25	2.4
25.0	25	26	29	27	1.2
50.0	33	24	32	30	2.8

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.741	0.2160	0.60415	2.0059	0.08488
1 - 4	-	4.914	0.0732	0.33244	1.1147	0.29107
1 - 5	-	5.056	0.0086	0.07946	0.2874	0.77834
1 - 6	-	5.071	0.0044	0.08531	0.3425	0.73645
1 - 7	-	5.055	0.0070	0.26029	1.1751	0.25447

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	30	18	25	24	3.5
MMS	500 µg/pl	-	156	155	165	159	3.2

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 16 - Experiment II - Pre-incubation method - WP2*uvr*A

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: WP2*uvr*A Titre: 288

Dose-level [µg/pl]	With metabolic activation Plate counts			Mean	S. E.
Untreated	42	36	33	37	2.6
0.00	34	31	29	31	1.5
156	33	22	31	29	3.4
313	34	30	28	31	1.8
625	29	24	22	25	2.1
1250	31 *	39 *	33 *	34	2.4
2500	22 *	30 *	33 *	28	3.3
5000	20 *	28 *	27 *	25	2.5

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	+	5.518	-0.0002	-0.07463	0.1980	0.84867
1 - 4	+	5.599	-0.0009	-0.51241	1.8869	0.08851
1 - 5	+	5.366	0.0002	0.21433	0.7912	0.44304
1 - 6	+	5.447	0.0000	-0.03081	0.1233	0.90342
1 - 7	+	5.492	-0.0001	-0.32123	1.4786	0.15564

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	50 µl/pl	+	34	26	36	32	3.1
2-Aminoanthracene	20 µg/pl	+	157	159	160	159	0.9

*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 17 - Experiment II - Pre-incubation method - TA98

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA98 Titre: 310

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	37	35	38	37	0.9	40	41	32	38	2.8
0.00	33	39	38	37	1.9	49	53	50	51	1.2
78.1	47	41	44	44	1.7	NT	NT	NT		
156	47	55	49	50	2.4	51	48	50	50	0.9
313	38	41	46	42	2.3	51	54	53	53	0.9
625	37	38	44	40	2.2	52	55	47	51	2.3
1250	39	34	35	36	1.5	43 *	46 *	42 *	44	1.2
2500	25 *	30 *	22 *	26	2.3	43 *	40 *	46 *	43	1.7
5000	NT	NT	NT			26 *	34 *	28 *	29	2.4

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	6.071	0.0067	0.89046	5.1772	0.00128
1 - 4	-	6.412	0.0010	0.27855	0.9172	0.38065
1 - 5	-	6.548	-0.0002	-0.09987	0.3619	0.72323
1 - 6	-	6.592	-0.0004	-0.42662	1.8868	0.07747
1 - 7	-	6.646	-0.0006	-0.78926	5.6025	0.00002
1 - 3	+	7.070	0.0004	0.43290	1.2706	0.24448
1 - 4	+	7.108	0.0001	0.19385	0.6249	0.54606
1 - 5	+	7.226	-0.0004	-0.66541	3.2140	0.00678
1 - 6	+	7.178	-0.0003	-0.75306	4.5782	0.00031
1 - 7	+	7.225	-0.0003	-0.92802	10.8590	0.00000

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	50 µl/pl	-	38	35	30	34	2.3
2-Nitrofluorene	2 µg/pl	-	145	141	134	140	3.2
DMSO	50 µl/pl	+	44	41	36	40	2.3
2-Aminoanthracene	2 µg/pl	+	422	439	450	437	8.1

* : Thinning of the background lawn
NT: Not tested

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 18 - Experiment II - Pre-incubation method - TA100

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: TA100 Titre: 268

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	167	161	130	153	11.5	140	161	148	150	6.1
0.00	160	163	142	155	6.6	155	156	148	153	2.5
78.1	132	145	133	137	4.2	NT	NT	NT		
156	130	128	123	127	2.1	143	133	120	132	6.7
313	126	134	123	128	3.3	131	111	121	121	5.8
625	128	*117	* 99 *	115	8.5	124	114	117	118	3.0
1250	109	*103	*105 *	106	1.8	133	*101	*107 *	114	9.8
2500	68	* 84	* 96 *	83	8.1	98	* 94	*105 *	99	3.2
5000	NT	NT	NT			92	* 97	*102 *	97	2.9

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	12.388	-0.0075	-0.86130	4.4849	0.00285
1 - 4	-	12.135	-0.0034	-0.72247	3.3044	0.00795
1 - 5	-	12.019	-0.0023	-0.76000	4.2162	0.00101
1 - 6	-	11.856	-0.0014	-0.80731	5.4721	0.00005
1 - 7	-	11.764	-0.0011	-0.88582	8.3211	0.00000
1 - 3	+	12.301	-0.0044	-0.85246	4.3142	0.00351
1 - 4	+	12.037	-0.0022	-0.77707	3.9041	0.00294
1 - 5	+	11.796	-0.0011	-0.67730	3.3193	0.00554
1 - 6	+	11.661	-0.0008	-0.77706	4.9382	0.00015
1 - 7	+	11.433	-0.0004	-0.74335	4.8441	0.00011

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	167	161	130	153	11.5
Sodium Azide	1 µg/pl	-	700	733	696	710	11.7
DMSO	50 µl/pl	+	114	117	126	119	3.6
2-Aminoanthracene	2 µg/pl	+	775	896	803	825	36.6

---

* : Thinning of the background lawnù
NT: Not tested

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 19 - Experiment III - Plate incorporation method - WP2*uvrA*

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: WP2*uvrA* Titre: 281

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.
Untreated	25	19	19	21	2.0
0.00	24	24	21	23	1.0
50.0	22	22	20	21	0.7
100	30	25	24	26	1.9
200	24	25	22	24	0.9
400	24	24	20	23	1.3
800	28 *	21 *	27 *	25	2.2

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.680	0.0033	0.48999	1.4872	0.18057
1 - 4	-	4.776	0.0008	0.25309	0.8273	0.42738
1 - 5	-	4.838	0.0000	-0.02761	0.0996	0.92220
1 - 6	-	4.807	0.0002	0.22713	0.9329	0.36474

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	25	19	19	21	2.0
MMS	500 µg/pl	-	155	152	160	156	2.3

---

*: Thinning of the background lawn

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 20 - Experiment III - Pre-incubation method - WP2*uvr*A

STUDY NO.: 52400

SOLVENT: Ethanol

Strain: WP2*uvr*A Titre: 281

Dose-level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.
Untreated	28	27	23	26	1.5
0.00	26	20	37	28	5.0
50.0	23	30	27	27	2.0
100	18	28	25	24	3.0
200	17	27	22	22	2.9
400	20	17	30	22	3.9
800	28 *	27 *	25 *	27	0.9

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	5.260	-0.0037	-0.29637	0.8210	0.43871
1 - 4	-	5.228	-0.0029	-0.41136	1.4272	0.18400
1 - 5	-	5.118	-0.0013	-0.34695	1.3338	0.20517
1 - 6	-	4.958	0.0000	-0.00095	0.0038	0.99701

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated	50 µl/pl	-	28	27	23	26	1.5
MMS	500 µg/pl	-	126	137	144	136	5.2

---

*: Thinning of the background lawn

[redacted]7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

APPENDIX 1 - Historical control data without metabolic activation

STUDY NO.: 52400

	Untreated Plate incorporation	Untreated Pre-incubation	Positive control Plate incorporation	Positive control Pre-incubation
TA1535				
Mean value	18	18	580	579
SD	2.6	2.5	80.0	79.3
n	129	131	129	131
TA1537				
Mean value	17	17	169	162
SD	2.1	1.8	37.3	47.6
n	128	132	128	132
TA98				
Mean value	32	32	187	181
SD	3.3	2.9	26.7	26.1
n	131	126	131	126
TA100				
Mean value	143	141	818	818
SD	16.0	16.1	122.1	125.3
n	131	130	131	130
WP2*uvrA*				
Mean value	28	28	184	219
SD	3.3	3.2	18.7	47.7
n	78	73	78	73

SD : standard deviation
n : number of experiments

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

APPENDIX 2 - Historical control data with metabolic activation

STUDY NO.: 52400

	Untreated Plate incorporation	Untreated Pre-incubation	Positive control Plate incorporation	Positive control Pre-incubation
TA1535				
Mean value	16	16	141	95
SD	1.8	1.9	30.3	10.4
n	129	128	129	128
TA1537				
Mean value	21	21	120	97
SD	2.6	2.2	21.4	12.4
n	128	133	128	133
TA98				
Mean value	39	40	625	671
SD	3.7	3.0	147.8	198.8
n	131	118	131	118
TA100				
Mean value	153	150	1278	1150
SD	16.1	15.1	198.8	198.0
n	132	130	132	130
WP2*uvr*A				
Mean value	35	34	203	192
SD	3.4	3.3	37.4	38.2
n	78	71	78	71

SD : standard deviation
n : number of experiments

[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

ADDENDUM I - Certificate of analysis

STUDY NO.: 52400

SOLVAY SOLEXIS

Centro Ricerche e Svilup
Viale Lombardia, 20
20021 Bollate (MI)

Pag.

Richiesta analisi Numero:

Data 23/11/2004

Risposta Numero:

Data

C.D.C.	***Commessa***	***Richiedente***	***Destinatario***
0316	N751	[redacted]	[redacted]

Prodotto, suoi riferimenti e precauzioni antinfortunistiche

[redacted] 7850 Acido Batch: 3223 ON

Tossico e corrosivo: utilizzare guanti e occhiali, lavorare sotto cappa

Lavoro richiesto

Caratterizzazione NMR: analisi terminali, peso molecolare

Risultati

Si allega spettro 19F-NMR con caratterizzazione del prodotto
Si nota la presenza di piccolissime quantità di estere

Distribuzione

Riferimenti 11542

Analitica e Strutturistica

Firme [redacted]

[redacted]
Page FLK7850_3223ON_
PFPE

exp2 s2pul

SAMPLE		DEC. & VT	
date	Nov 30 2004	dfrq	299.917
solvent	Acetone	dn	H1
file	/space/MYFID8~	dpwr	30
	[redacted]/FLK7850~	dof	0
	_3223ON_001.fid	dm	nnn
ACQUISITION		dmm	c
sfrq	282.176	dmf	200
tn	F19	PROCESSING	
at	0.500	lb	2.00
np	75170	lsfid	-1
sw	75188.0	wtfile	
fb	41400	proc	lp
bs	10	fn	131072
tpwr	61		
pw	4.5	werr	reset
d1	2.000	wexp	procplot
tof	2400.0	wbs	
nt	1000	wnt	
ct	1000		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-45146.6		
wp	39502.5		
vs	96		
sc	0		
wc	250		
hzmm	150.01		
is	483.57		
rfl	22965.4		
rfp	-39578.8		
th	0		
ins	140.895		
ai	ph		

IS=484

FLK7850_3223ON_001.jpeg

SOLVAY BOLLATE NMR 300 SOLEXIS

[redacted]
Page FLK7850_3223ON_
PFPE

Pulse Sequence: s2pul

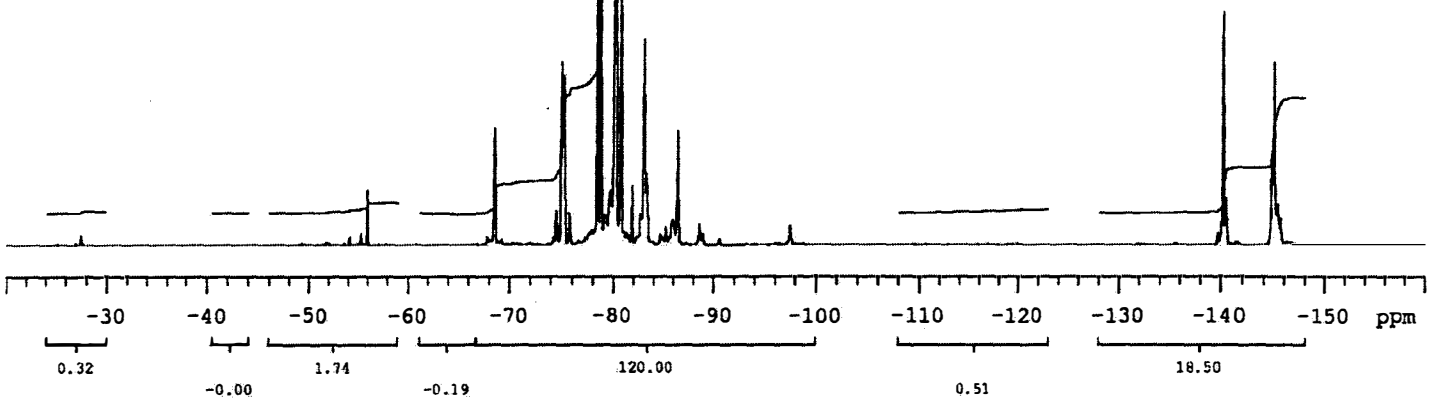
MOL.WEIGHT = 551

EQUIV.WEIGHT = 551

C1=1.0 % C3 "-OCF2O-"

C2=9.1 % C3 "-OCF(CF3)O-"

-OCF3	=	3.3
-OCFCF3COOH	=	0.8
-OCF2CL	=	1.1
-OCF(CF3)CF2CL	=	15.8
-OCF2CF(CF3)CL	=	29.7
-OCF2COOH	=	49.2



[redacted] 7850:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

ADDENDUM II - Study Protocol

STUDY NO.: 52400

RESEARCH TOXICOLOGY CENTRE - ROMA

[redacted]7850

BACTERIAL MUTATION ASSAY

(S. typhimurium and E. coli)

Final Protocol
prepared for

SOLVAY SOLEXIS S.p.A.
Viale Lombardia, 20
20021 Bollate (MI)
Italy

by

RESEARCH TOXICOLOGY CENTRE S.p.A.
Via Tito Speri, 12
00040 Pomezia (Roma)
Italy

RTC Enquiry Number: 52400

January 2006

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Reg. Soc. Trib. di Roma n° 2828/72
Cod. Fisc.: 00853120584
Partita IVA: 00920611001

BACTERIAL MUTATION ASSAY
(S. typhimurium and E. coli)

MANAGEMENT OF STUDY

Scientific Director	:	[redacted]
Head of Genetic and Cellular Toxicology	:	[redacted]
Study Director	:	[redacted]
Sponsor	:	SOLVAY SOLEXIS S.p.A. Viale Lombardia, 20 20021 Bollate (MI) Italy
Monitor	:	[redacted]

QUALITY ASSURANCE

Quality Assurance Manager	:	[redacted]

LOCATION OF STUDY

The study will be performed at	:	Research Toxicology Centre S.p.A. Via Tito Speri, 12 00040 Pomezia (Roma) Italy

The laboratory facilities, archives and administration are located at this site.

TIME SCHEDULE OF STUDY

The Study will be conducted with a time schedule agreed between the Sponsor and RTC.

TEST ITEM IDENTITY

The test item will be : [redacted] 7850

BACTERIAL MUTATION ASSAY
(*S. typhimurium and E. coli*)

1. INTRODUCTION

1.1 Objective

To assay the test item for the ability to induce gene mutations in *Salmonella_typhimurium* and *Escherichia coli*, as measured by reversion of auxotrophic strains to prototrophy.

1.2 Regulatory requirements

This study will be conducted in compliance with the GLP regulations of:

- US FDA [21 CFR part 58, 22 December 1978] and subsequent revisions;
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004;
- ENV/MC/CHEM(98)17 "OECD principles on Good Laboratory Practice – as revised in 1997";
- Decreto Legislativo no. 120 of 27 January 1992 and subsequent revisions.

In addition, the study is designed to comply with the experimental methods indicated in the guidelines of:

- EEC Council Directive 2000/32, Annex 4D.
- OECD Guidelines for the testing of chemicals No. 471 (Adopted July 1997).
- ICH S2A Genotoxicity: Specific Aspects of Regulatory Tests, Step 5.

1.3 Principles of the method

Reverse mutation assays employ bacterial strains which are already mutant at a locus whose phenotypic effects are easily detected. The *Salmonella* tester strains have mutations causing dependence on a particular amino acid (histidine) for growth. The ability of test items to cause reverse mutations (reversions) to histidine-independence can easily be measured. The *E. coli* tester strains of the WP2 series are similarly mutant at the tryptophan locus.

Since many chemicals only demonstrate mutagenic activity after metabolism to reactive forms, in order to detect these "indirect mutagens" the test is performed in the presence and absence of a rat liver metabolising system.

2. TEST ITEM

2.1 It is the responsibility of the Sponsor to supply the test item, accompanied by analytical data confirming the identity, purity, stability, strength and composition of the substance, the solubility and stability in the proposed vehicle and details of any known hazards to laboratory staff. The test item should be accompanied by a certificate of analysis.

2.2 Approximately one year after the submission of the final report, remaining amounts of the test item will be destroyed by incineration. An aliquot of the substance will be retained within the archives of the testing facility for a period of ten years after which it will be destroyed.

2.3 The test item identity is indicated on previous pages of this protocol.

2.4 Unless otherwise indicated by the Sponsor the storage conditions for the test item will be room temperature.

2.5 The precautions necessary when handling either the test item or prepared formulations of the test item are based on information supplied by the Sponsor. The minimum safety precautions necessary are detailed under the RTC Hazard Classification System, according to RTC standard procedures.

2.6 The amount of test item received and used will be recorded according to standard procedures.

2.7 Fresh solutions of the test item will be prepared for each day's work; solutions will be prepared on a weight/volume basis without correction for the displacement due to the volume occupied by the test item. Unless specified by the Sponsor, concentrations of solutions will be expressed in terms of material as received, and not of active constituents. Preferred solvents will be sterile distilled water, culture medium, DMSO, ethanol, acetone. Other solvents may be used as necessary.

2.8 No assay of test item stability, nor its concentration and homogeneity in the solvent/vehicle will be undertaken, nor samples of formulated test item consigned to the Sponsor, without express instructions from the Sponsor. No determination of the absorption of the test item in the test system will be made without express instructions from the Sponsor.

3. MATERIALS

3.1 Bacterial strains

Stocks of *Salmonella* tester strains (TA 1535, TA 1537, TA 1538, TA 98, TA 100, TA 97 and TA 102 and some other related strains) were obtained from [redacted] University of California. Stocks of *E. coli* tester strains (WP2, WP2 *uvr*A and WP2 *uvr*A pKM101) were obtained from Life Science Research, Occold, Suffolk, UK. Permanent stocks are kept at -80°C, and overnight subcultures of these stocks are prepared for each day's work. The cultures used in each experiment contain a high titre of viable bacteria (1-5 x 10^9 cells per ml). The titre of each tester strain culture will be demonstrated in each experiment through the determination of viable cell numbers using nutrient agar plates.

The presence of the appropriate genetic markers in these strains is checked on a monthly basis for those in regular use, and as necessary for other strains, as follows:

Histidine requirement	:	No Growth on Minimal plates + Biotin. Growth on Minimal plates + Biotin + Histidine.
Tryptophan requirement	:	No Growth on Minimal agar plates Growth on Minimal plates + Tryptophan.
*uvr*A, *uvr*B	:	Sensitivity to UV irradiation.
rfa	:	Sensitivity to Crystal Violet.
pKM101	:	Resistance to Ampicillin.

Strain identity is also confirmed by reference to the spontaneous reversion levels and responses to mutagens during use. Bacterial cultures in liquid and on agar are clearly identified with their identity.

Detailed information about the genetic constitution of the tester strains may be found in the cited publications of [redacted] and [redacted] and [redacted]

3.2 Media

The following growth media will be used:

Nutrient Broth: Oxoid Nutrient Broth No 2 will be prepared at a concentration of 2.5% in distilled water and autoclaved prior to use.
This will be used for the preparation of liquid cultures of the tester strains.

Nutrient Agar: Oxoid Nutrient Broth No 2 (25g) and Difco Bacto-agar (15g) will be added to one litre of distilled water and autoclaved.
The solution will then be poured into plastic Petri dishes and allowed to solidify and dry before use. These plates will be used for the non-selective growth of the tester strains. Incubations on Nutrient Agar will be for approximately 48 or 72 hours.

Minimal Agar: Minimal medium agar will be prepared as 1.5% Difco Bacto-agar in Vogel-Bonner Medium E, with 2% Glucose, and poured into plastic Petri dishes.

Top Agar: "Top Agar" (overlay agar) will be prepared as 0.6% Difco Bacto-agar + 0.5% NaCl in distilled water. This solution will be autoclaved, and stored. Prior to use 10 ml of a sterile solution of 0.5 mM Biotin + 0.5 mM Histidine (or 0.5 mM tryptophan) will be added to 100 ml of the top agar.

All incubations will be at 37°C.

3.3 S9 mix

The S9 liver tissue fraction will be prepared according to RTC standard procedures or will be obtained from an appropriate supplier (MOLTOX, Molecular Toxicology, Inc., USA). Induction of drug metabolising enzyme-levels is routinely performed using phenobarbitone and betanaphthoflavone (Mixed Induction); induction with Aroclor 1254 will be performed if specifically requested by the Sponsor. Records pertaining to the preparation of the S9 fraction are kept on file at RTC. The mixture of S9 tissue fraction and cofactors (S9 mix) will be prepared as follows (for each 10 ml):

S9 tissue fraction	1.0 ml
NADP (100 mM)	0.4 ml
G-6-P (100 mM)	0.5 ml
KCl (330 mM)	1.0 ml
MgCl2 (100 mM)	0.8 ml
Phosphate buffer (pH 7.4, 200 mM)	5.0 ml
Distilled Water	1.3 ml
	10.0 ml

3.4 Control items

Positive control treatments will be used in each experiment. The positive control agents are obtained commercially and characterised by their labelling, and their stability determined from the scientific literature. Sodium azide and methylmethanesulphonate will usually be dissolved in distilled water; 9-aminoacridine, 2-nitrofluorene and 2-aminoanthracene will usually be dissolved in DMSO. Determination of the stability and concentration of solutions of these agents will not be undertaken since it is sufficient to provide evidence for the correct expected response of the test system to them.

4. PRELIMINARY TOXICITY TEST

4.1 Experimental design

In order to establish the concentrations of test item to be used in the main assay, a preliminary toxicity test will be performed.
This test follows the method described in section 6.1, using only one plate per dose level, a single S9 mix concentration (10%) and covering a wide range of concentrations of the test item.

The highest dose-level for this preliminary test, unless limited by the solubility of the test item, will be 5 mg/plate, and the lower dose-levels will be spaced at approximately half-log intervals.

4.2 Selection of dose-levels

The toxicity will be assessed on the basis of a decline in the number of spontaneous revertants or a thinning of the background lawn. The highest dose-level for the mutation assays will be selected as a concentration which elicits moderate toxicity. If there is no evidence of toxicity following treatment with the test item, then the highest dose-level will be 5 mg/plate.

5. EXPERIMENTAL DESIGN

Each experiment will include solvent/vehicle and positive controls, and at least five doses of the test item, tested in the absence and presence of an S9 metabolising system. Three replicate plates will be used at each test point. If a first experiment produces a clear positive response, no further experiments will be undertaken.
If negative or equivocal results are obtained in the first experiment a confirmatory experiment will be performed using the pre-incubation method. A further experiment may be undertaken if inconsistent results are obtained. The five bacterial strains *S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2 *uvr*A will be used in this study.

Solvent/vehicle controls: untreated and solvent vehicle controls will be prepared for each experiment; when the solvent is distilled water, these will be considered to be equivalent and only one set of controls will be performed.

Positive controls: treatments are indicated in the following table:

Tester strain	*Absence of S9*	*Presence of S9*
TA1535	sodium azide 1 µg/plate	2-aminoanthracene 1 µg/plate
TA100	sodium azide 1 µg/plate	2-aminoanthracene 1 µg/plate (2 µg/plate)
TA1537	9-amino-acridine 50 µg/plate	2-aminoanthracene 1 µg/plate
TA98	2-nitrofluorene 2 µg/plate	2-aminoanthracene 1 µg/plate (2 µg/plate)
WP2 *uvr*A	methylmethanesulphonate 500 µg/plate	2-aminoanthracene 10 µg/plate (20 µg/plate)

Concentrations refer to both treatment methods. When two values are given, the figures in brackets refer to the pre-incubation method assay.

Test item: the highest dose-level of the test item to be used will be selected as described above. Further dose levels will be selected at intervals of a factor of two.

Where it seems advisable, further test points or controls may be included in experiments.

In addition, plates will be prepared to check the sterility of the test item solutions and the S9 mix, and dilutions of the bacterial cultures will be plated on nutrient agar plates to establish the number of bacteria in the cultures.

6. ASSAY PROCEDURE

6.1 Plate-incorporation

The components of the assay (the tester strain bacteria, the test item and S9 mix or phosphate buffer) will be added to molten overlay agar and vortexed. The mixture will then be poured on the surface of a minimal medium agar plate, and allowed to solidify prior to incubation.

The overlay mixture will be composed as follows:

(i)	Overlay agar (held at 45°C)	2 ml
(ii)	Test or control item solution	0.1 ml
(iii)	S9 mix or phosphate buffer	0.5 ml
(iv)	Bacterial suspension	0.1 ml

The volume of test item solution, as indicated, will usually be 0.1 ml; in the event that it is necessary to alter this volume, the quantities used will be carefully recorded.

6.2 Pre-incubation

The components will be added in turn to an empty test-tube:

(i) Bacterial suspension	0.1 ml
(ii) Test or control item solution	0.05 ml
(iii) S9 mix or phosphate buffer (Ph 7.4, 0.1 M)	0.5 ml

The volume of test item solution, as indicated, will usually be 0.05 ml. Where control or test items are dissolved in aqueous solvents, the volume used may be 0.1 ml. In the event that it is necessary to alter this volume, the quantities used will be carefully recorded.

The treatment mixture will be vortexed and placed at 37°C for 30 minutes. Two ml of overlay agar will then be added and the mixture vortexed again and poured onto the surface of a minimal medium agar plate and allowed to solidify.

6.3 Incubation and scoring

The prepared plates will be inverted and incubated for approximately 72 hours at 37°C. When the test item is a liquid at ambient temperature, the plates will be incubated in separate closed containers for each dose-level. After this period of incubation, the plates may be held at 4°C prior to scoring. Scoring is effected by counting the number of revertant colonies on each plate, either manually, or using a Cardinal - Automatic colony counting system (Perceptive Instruments). Contaminated plates will be considered on a case-by-case basis.

7. REPORTING

7.1 Presentation of data

The data will be presented in tabular form. The individual plate counts for each experiment will be given, together with the means and standard errors of the means, and regression analyses.

7.2 Evaluation of data

For the test item to be considered mutagenic, two-fold (or more) increases in mean revertant numbers must be observed at two consecutive dose-levels or at the highest practicable dose-level only. In addition there must be evidence of a dose- response relationship showing increasing numbers of mutant colonies with increasing dose-levels.
Evaluation of Ames test data based on a 'doubling rate' has been shown to be as effective as statistical techniques in allowing the correct interpretation of test results (Chu et al. 1981).

7.3 Reporting procedure

A Draft Report will be supplied, and a Final Report issued subsequently to include any agreed changes or amendments.
If any corrections or additions are required to the Final Report, these will be in the form of an amendment by the Study Director. The amendment will clearly identify that part of the Final report that is being added to or corrected, and the reasons for the changes, and will be signed and dated by the person responsible.

7.4 Final report

The following information and data will be included in the final report:

- name and address of the facility performing the study and the dates on which the study was initiated and completed;
- objective and procedures stated in the approved protocol, including approved changes to the original protocol;
- the test article, identified by name, chemical name or chemical number;
- method used;
- any unforeseen circumstances that may have affected the quality or integrity of the study;
- data generated while conducting the study;
- statistical methods employed for analysing the data;
- a summary of the data, an analysis of the data and a statement of the conclusions drawn from the analysis;
- historical untreated and positive control data;
- the name and signature of the Study Director;
- the location where all raw data, specimens and final report are to be stored;
- Quality Assurance statement.

One original unbound, one copy bound and a PDF version will be supplied.

7.5 Records kept

Full records will be maintained of all aspects of study conduct, along with the results of all measurements and observations. Prior to final archiving of the study data a full list will be prepared of all records associated with the study.

7.6 Archiving

All raw data, records and documentation arising from this study and a copy of the final report consigned, generated during the course of this study will be retained at RTC. Archiving will be provided for a period of 3 years after which the Sponsor will be contacted for instructions regarding despatch or disposal of the material. As a further option, archiving space can be rented for an additional time. The signed Final Protocol and the top copy of the Final Report will be despatched to and archived by the Sponsor.

8. STUDY CONDUCT

8.1 Language

English language and Italian language versions of the study protocol, Standard Operating Procedures and other study documents may be used interchangeably. Similarly, English and Italian renderings of chemical names, including that of the test material will be considered to be equivalent.

8.2 Scientific decisions

The procedures described in this protocol may not comprehensively cover all the circumstances that can arise in the assay of test items. When the study director considers it advisable to modify the procedures described for the selection of a solvent, selection of dose-levels, interpretation of the outcome of the study or other aspects of the study conduct, he will record carefully the decision he has reached and the reasoning which led to it.

8.3 Quality assurance

The study is subjected to the procedure for quality assurance as defined by the relevant GLP regulations. Specifically:

- the protocol is inspected for compliance;
- procedures of the laboratories concerned will be inspected at intervals adequate to assure the integrity of the study;
- the final report is reviewed to ensure that it accurately describes the methods and relevant Standard Operating Procedures and that the results are in agreement with the raw data;
- periodic reports on these activities are made to management and the Study Director.

All raw data pertaining to the study will be available for inspection by the study monitor (for scientific monitoring) or the Quality Assurance Unit of the Sponsor (compliance monitoring).

9. DEPARTURES FROM REGULATORY REQUIREMENTS

Items which are the responsibility of the Sponsor are indicated in sections 2.1, 2.4, 2.5, 2.7, 2.8 and 3.3 of this protocol. Since full compliance with regulatory requirements may depend on the performance of these items, the Sponsor should ensure that appropriate actions are initiated or undertaken.

10. REFERENCES

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Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test.
Mutation Research 31, 347-364.

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Evaluating statistical analyses and reproducibility of microbial mutagenicity assays.
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Guide for the *Salmonella typhimurium*/mammalian microsome tests for bacterial mutagenicity.
Mutation Research 189, 83-91.

Gatehouse D. et al. (1994)
Recommendations for the performance of bacterial mutation assays.
Mutation Research 312, 217-233.

Green M.H.L. and W.J. Muriel (1976)
Mutagen testing using TRP^+ reversion in *Escherichia coli*.
Mutation Research 38, 3-32.

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

Venitt S., R. Forster and E. Longstaff (1983).
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in: Report of the UKEMS Subcommittee on Guidelines for Mutagenicity testing.
U.K.E.M.S., Swansea, 1983.

Venitt S., C. Croften-Sleigh and R. Forster (1984)
Bacterial mutation assays using reverse mutation
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S. Venitt and J.M. Parry (eds.)
IRL Press, Oxford, 1984.

APPROVAL PAGE

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