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SYN545682

SYN545682 - Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay

Final Report

DATA REQUIREMENT(S): OECD 471 (1997)
2000/32/EC (2000)
EPA OPPTS 870.5100 (1998)

AUTHOR(S): Andrea Sokolowski

STUDY COMPLETION DATE: June 19, 2008

PERFORMING LABORATORY: RCC
Cytotest Cell Research GmbH (RCC-CCR)
In den Leppsteinswiesen 19
64380 Rossdorf, Germany

LABORATORY PROJECT ID: Report Number: 1179000
Study Number: 1179000
Task Number: T012727-05

SPONSOR: Syngenta Ltd
Jealott's Hill International Research Centre
Bracknell, Berkshire RG42 6EY United Kingdom

STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Number:	1179000
Test Item:	SYN545682
Study Director:	Dipl. Biol. Andrea Sokolowski
Title:	SYN545682 - Salmonella typhimurium and Escherichia coli Reverse Mutation Assay

This study performed in the test facility of RCC Cytotest Cell Research GmbH; In den Leppsteinswiesen 19, 64380 Rossdorf, Germany was conducted in compliance with Good Laboratory Practice Regulations:

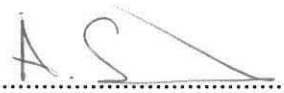
"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002.

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final]

There were no circumstances that may have affected the quality or integrity of the study.

R C C - C C R
In den Leppsteinswiesen 19
64380 Rossdorf, Germany

Dipl. Biol. Andrea Sokolowski
Study Director



Date: June 19, 2008

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Number: 1179000

Test Item: SYN545682

Study Director: Dipl. Biol. Andrea Sokolowski

Title: SYN545682 - Salmonella typhimurium and Escherichia coli Reverse Mutation Assay

The general facilities and activities of RCC Cytotest Cell Research GmbH are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were inspected periodically. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	May 09, 2008	May 09, 2008
Process Inspection (preparation for the application):	May 26, 2008	May 26, 2008
Report:	June 13, 2008	June 13, 2008

This statement is to confirm that the present report reflects the raw data.

Head of Quality Assurance Unit Frauke Hermann

F. Hermann

.....

Date: June 19, 2008

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Dipl. Biol. Andrea Sokolowski	Study Director
Dr. Hans-Eric Wollny	Deputy Study Director
Dr. Wolfgang Völkner	Management
Frauke Hermann	Head of Quality Assurance Unit
Ewan Booth	Syngenta Study Manager

Test Facility: RCC
Cytotest Cell Research GmbH (RCC-CCR)
In den Leppsteinswiesen 19
64380 Rossdorf
Germany

Contracting Institute: RCC Ltd
4452 Itingen
Switzerland

Reference Number: B96805

Study dates

Study initiation date: 09 May 2008
Experimental start date: 19 May 2008
Experimental termination date: 27 May 2008

Deviations from the guidelines

None

Retention of samples

Raw data and a sample of the test item.

Performing laboratory test substance reference number

S 8785 11

GENERAL INFORMATION (CONTINUED)

Other

RCC Cytotest Cell Research GmbH will archive the following data for 15 years:

Raw data, study plan, report, and a sample of the test item.

No data will be discarded without the sponsor´s consent.

Good laboratory practice

The study was performed in compliance with:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final]

Deviations to study plan

There were no deviations to the study plan.

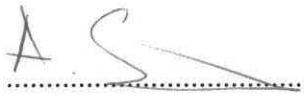
Distribution of the report

Sponsor	2 × electronic copy (1 × pdf-file, 1 × Word file)
Study Director	1 × (original)

Project staff signatures

Study Director

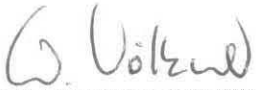
Dipl. Biol. Andrea Sokolowski



Date: June 19, 2008

Management

Dr. Wolfgang Völkner



Date: June 19, 2008

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1.0 EXECUTIVE SUMMARY

1.1 Study design

This study was performed to investigate the potential of SYN545682 to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strains WP2 uvrA (pKM 101), and WP2 (pKM 101).

1.2 Results

The plates incubated with the test item showed normal background growth with and without metabolic activation in all strains in both experiments.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation in experiment I. In experiment II, minor toxic effects were observed in strain TA 1537 at 1000 and 5000 µg/plate with metabolic activation. No toxic effects were observed in the remaining strains.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with SYN545682 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

1.3 Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, SYN545682 is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli reverse mutation assay.

2.0 INTRODUCTION

2.1 Purpose

The experiments were performed to assess the potential of the test item to induce gene mutations by means of two independent Salmonella typhimurium and Escherichia coli reverse mutation assays. Experiment I was performed as a plate incorporation assay. Since a negative result was obtained in this experiment, experiment II was performed as a pre-incubation assay.

The most widely used assays for detecting gene mutations are those using bacteria (3). They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency with which an agent reverses or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers, are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to growth in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The S. typhimurium histidine (his) and the E. coli tryptophan (trp) reversion system measures $his^- \rightarrow his^+$ and $trp^- \rightarrow trp^+$ reversions, respectively. The S. typhimurium and Escherichia coli strains are constructed to differentiate between base pair (TA 1535, TA 100, WP2 uvrA (pKM 101), and WP2 (pKM 101) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test item with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect eight dose levels with adequately spaced intervals were tested. The maximum dose level was 5000 µg/plate.

To validate the test, reference mutagens were tested in parallel to the test item.

2.2 Regulatory guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

"Ninth Addendum to OECD Guidelines for Testing of Chemicals", Section 4, No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997

"Commission Directive 2000/32/EC, L1362000, Annex 4D", dated May 19, 2000

"US EPA Health Effects Test Guideline OPPTS 870.5100 (1998). Bacterial Reverse Mutation Test."

3.0 MATERIALS AND METHODS

3.1 Test item

Internal RCC-CCR Test Item Number: S8785 11

The test item and the information concerning the test item were provided by the sponsor.

Identity:	SYN545682
Batch No.:	BPS 1266/1
Purity:	98.4 %
Storage:	At room temperature
Stability in Solvent:	Not indicated by the sponsor
Reanalysis Date:	End of April, 2010

On the day of the experiment, the test item SYN545682 was dissolved in DMSO (MERCK, D-64293 Darmstadt; purity > 99 %). The solvent was chosen because of its solubility properties and its relative non-toxicity to the bacteria (5).

3.2 Controls

3.2.1 Negative controls

Concurrent untreated and solvent controls were performed.

3.2.2 Positive control substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Supplier:	SERVA, D-69042 Heidelberg
Catalogue No.:	30175
Purity:	at least 99 %
Dissolved in:	water deionised
Concentration:	10 µg/plate

Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	N 9504
Purity:	> 99.9 %
Dissolved in:	DMSO (MERCK, D-64293 Darmstadt; purity > 99 %)
Concentration:	10 µg/plate in TA 98, 50 µg/plate in TA 1537

Strains:	WP2 uvrA (pKM 101), WP2 (pKM 101)
Name:	methyl methane sulfonate, MMS
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue No.:	820775
Purity:	> 99.0 %
Dissolved in:	water deionised
Concentration:	3 µL/plate

3.2.2.1 With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA (pKM 101), WP2 (pKM 101)
Name:	2-aminoanthracene, 2-AA
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	A 1381
Purity:	97.5 %
Dissolved in:	DMSO (MERCK, D-64293 Darmstadt, purity > 99 %)
Concentration:	2.5 µg/plate (TA 1535, TA 1537, TA 98, TA 100), 10 µg/plate (WP2 uvrA (pKM 101), WP2 (pKM 101))

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range will be sufficient evidence of biological stability.

3.3 Experimental design

3.3.1 Characterisation of the Salmonella typhimurium and E. coli strains

The histidine dependent strains are derived from S. typhimurium strain LT2 through mutations in the histidine locus. Additionally due to the "deep rough" (rfa^-) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named $uvrB^-$. In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker (6).

Strain WP2 (4) and its derivatives all carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (Trp^+) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another. Additionally, the uvrA derivative is deficient in the DNA repair process (excisable repair damage). Such a repair-deficient strain may be more readily mutated by agents. The E. coli strain WP2 uvrA pKM101 and WP2 pKM101 are constructed by introduction of the R-factor plasmid pKM101.

When summarised, the mutations of the TA and E. coli strains used in this study can be described as follows:

Salmonella typhimurium		
Strains	**Genotype**	**Type of mutations indicated**
TA 1537	his D 6610; rfa^-; $uvrB^-$, R-factor	frame shift mutations
TA 98	his D 3052; rfa^-; $uvrB^-$; R-factor	" "
TA 1535	his G 46; rfa^-; $uvrB^-$:	base-pair substitutions
TA 100	his G 46; rfa^-; $uvrB^-$;R-factor	" "
Escherichia coli		
WP2 uvrA pKM101	trp E 56 $uvrA^-$:, R-factor	base-pair substitutions and others
WP2 pKM101	trp E 56:, R-factor	" "

Regular checking of the properties of the Salmonella typhimurium and E. coli strains regarding the membrane permeability and ampicillin resistance as well as normal spontaneous mutation rates is performed in RCC Cytotest Cell Research GmbH according to B. Ames et al. (1) and D. Maron and B. Ames (6). In this way it is ensured that the experimental conditions set down by Ames are fulfilled.

The bacterial strains TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA (pKM 101), and WP2 (pKM 101) were obtained from Trinova Biochem GmbH (35394 Gießen, Germany).

3.3.2 Storage

The strain cultures were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK, D-64293 Darmstadt) in liquid nitrogen.

3.3.3 Precultures

From the thawed ampoules of the strains 0.5 mL bacterial suspension was transferred into 250 mL Erlenmeyer flasks containing 20 mL nutrient medium. A solution of 20 µL ampicillin (25 µg/mL) was added to the strains TA 98, TA 100, WP2 uvrA (pKM 101), and WP2 (pKM 101). This nutrient medium contains per litre:

8 g Merck Nutrient Broth (MERCK, D-64293 Darmstadt)
5 g NaCl (MERCK, D-64293 Darmstadt)

The bacterial cultures were incubated in a shaking water bath for 4 hours at 37 °C.

3.3.4 Selective agar

The plates with the selective agar were obtained from E. Merck, D-64293 Darmstadt.

3.3.5 Overlay agar

The overlay agar contains per litre:

for Salmonella strains:	for Escherichia coli:
6.0 g MERCK Agar Agar*	6.0 g MERCK Agar Agar*
6.0 g NaCl*	6.0 g NaCl*
10.5 mg L-Histidine×HCl×H_2O*	2.5 mg Tryptophan*
12.2 mg Biotin*	

* (MERCK, D-64293 Darmstadt)

Sterilisations were performed at 121 °C in an autoclave.

3.4 Mammalian microsomal fraction S9 Mix

The bacteria used in this assay do not possess the enzyme systems which, in mammals, are known to convert promutagens into active DNA damaging metabolites. In order to overcome this major drawback an exogenous metabolic system is added in form of mammalian microsome enzyme activation mixture.

3.4.1 S9 (Preparation by R C C - C C R)

Phenobarbital/β-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar HanIbm rats, weight approx. 220 - 320 g induced by applications of 80 mg/kg b.w. Phenobarbital i.p. (Desitin; D-22335 Hamburg) and β-Naphthoflavone p.o. (Aldrich, D-89555 Steinheim) each on three consecutive days. The livers are prepared 24 hours after the last treatment. The S9 fractions are produced by dilution of the liver homogenate with a KCl solution (1/4, v/v) followed by centrifugation at 9000 g. Aliquots of the supernatant are frozen and stored in ampoules at -80 °C. Small numbers of the ampoules can be kept at -20 °C for up to one week. Each batch of S9 mix is routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

The protein concentration in the S9 preparation was 32.5 mg/mL (lot no. R010208) in both experiments.

3.4.2 S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 10% v/v in the S9 mix. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix:

8 mM	$MgCl_2$
33 mM	KCl
5 mM	Glucose-6-phosphate
5 mM	NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al.(1).

3.5 Pre-Experiment for toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with strains TA 1535, TA 1537, TA 98, TA 100, and WP2 uvrA pKM 101, and WP2 pKM 101. Eight concentrations were tested for toxicity and mutation induction with three plates each. The experimental conditions in this pre-experiment were the same as described below for the experiment I (plate incorporation test).
Toxicity of the test item results in a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.
The pre-experiment is reported as part of the main experiment I, if the following criteria are met:
Evaluable plates (>0 colonies) at five concentrations or more in all strains used.

3.6 Dose selection

In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. The pre-experiment is reported as part of experiment I. Since only minor toxic effects were observed 5000 µg/plate was chosen as the maximal concentration. Based on the precipitation of the test item, eight concentrations were also tested in experiment II.

The concentration range included two logarithmic decades. The following concentrations were tested in experiment II:

3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

3.7 Experimental performance

For each strain and dose level including the controls, three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µL	Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µL	S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL	Bacteria suspension (cf. test system, pre-culture of the strains),
2000 µL	Overlay agar

In the pre-incubation assay 100 µL test solution, 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension was mixed in a test tube and shaken at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on selective agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark (2).

3.8 Data evaluation

3.8.1 Data recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, Suffolk CB 7BN, UK) with the software program Ames Study Manager. The counter was connected to an IBM AT compatible PC with printer to print out the individual values and the means from the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results). Due to precipitation of the test item, the colonies were partly counted manually.

3.8.2 Acceptability of the assay

The Salmonella typhimurium and Escherichia coli reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of our historical data
- the positive control substances should produce a significant increase in mutant colony frequencies

3.8.3 Evaluation of results

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control is observed (3).

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration (2).

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

3.8.4 Biometry

According to the OECD guideline 471, a statistical analysis of the data is not mandatory.

4.0 RESULTS AND DISCUSSION

4.1 Dose selection

In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. The pre-experiment is reported as part of experiment I. Since no toxic effects were observed 5000 µg/plate was chosen as the maximal concentration. Based on the precipitation of the test item, eight concentrations were also tested in experiment II.

The concentration range included two logarithmic decades. The following concentrations were tested in experiment II:

3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

4.2 Discussion

The test item SYN545682 was assessed for its potential to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strains WP2 uvrA (pKM 101) and WP2 (pKM 101).

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth with and without metabolic activation in all strains in both experiments.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation in experiment I. In experiment II, minor toxic effects were observed in strain TA 1537 at 1000 and 5000 µg/plate with metabolic activation. No toxic effects were observed in the remaining strains.

Precipitation of the test item was observed in the test tubes from 1000 up to 5000 µg/plate with and without metabolic activation in experiment I. Precipitation was also observed on the agar plates from 1000 up to 5000 µg/plate with and without metabolic activation in experiment I, and from 333 up to 5000 µg/plate without metabolic activation and 1000 up to 5000 µg/plate with metabolic activation in experiment II.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with SYN545682 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

The laboratory´s historical control range was exceeded in the untreated control of strain WP2 pKM 101 with and without metabolic activation in experiment II. This elevated colony count was considered to be the result of biologically irrelevant fluctuations and had no detrimental impact on the outcome of the study. The current historical range, is based on a limited number of experiments with these strains and is still in the process of being developed. Therefore, excursions outside the laboratory´s historical control range are not unexpected.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

5.0 CONCLUSION

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, SYN545682 did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

6.0 REFERENCES

1. Ames, B.N., J. McCann, and E. Yamasaki (1977)
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   Mutation Res. 64, 159-165

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   Mutation Res. 65, 133-226

4. Green, M.H.L. and W.J. Muriel (1976)
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   Mutation. Res. 38, 3- 32

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   Compatibility of organic solvents with the Salmonella/Microsome Test
   Mutation Res. 88, 343-350

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

7. Watanabe K., Sasaki, T., Kawakami, K. (1998)
   Comparisons of chemically-induced mutation among for bacterial strains, Salmonella typhimurium TA 102 and TA 2638 and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101:collaborative study III and evaluation of the usefulness of these strains
   Mutation Res. 416, 169-181

TABLES SECTION

TABLE 1. Summary of Results Pre-Experiment/Experiment I

Study Name: 1179000 Study Code: RCC-CCR 1179000
Experiment: 1179000 VV Plate Date Plated: 19/05/2008
Assay Conditions: Date Counted: 26/05/2008

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA pkm 101	WP2pKm 101
Without Activation	DMSO		15 ± 5	11 ± 2	22 ± 1	121 ± 8	390 ± 11	267 ± 18
	Untreated		17 ± 3	10 ± 4	28 ± 5	116 ± 5	389 ± 38	248 ± 16
	SYN545682	3 µg	16 ± 4	13 ± 4	23 ± 1	117 ± 19	377 ± 12	235 ± 8
		10 µg	17 ± 3	13 ± 3	23 ± 4	116 ± 8	383 ± 5	276 ± 7
		33 µg	11 ± 3	13 ± 3	24 ± 3	109 ± 13	365 ± 21	254 ± 12
		100 µg	15 ± 5	12 ± 4	21 ± 4	122 ± 8	386 ± 21	268 ± 54
		333 µg	17 ± 7	14 ± 1	27 ± 3	123 ± 10	354 ± 1	236 ± 14
		1000 µg	17 ± 3 P M	11 ± 3 P M	22 ± 3 P M	112 ± 10 P M	355 ± 14 P M	238 ± 9 P M
		2500 µg	12 ± 2 P M	10 ± 3 P M	17 ± 3 P M	111 ± 9 P M	352 ± 10 P M	239 ± 12 P M
		5000 µg	9 ± 3 P M	10 ± 3 P M	17 ± 2 P M	103 ± 5 P M	347 ± 24 P M	229 ± 9 P M
	NaN3	10 µg	1875 ± 89			2212 ± 87		
	4-NOPD	10 µg			390 ± 9			
	4-NOPD	50 µg		85 ± 5				
	MMS	3.0 µL					4357 ± 292	3837 ± 240

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 1. Summary of Results Pre-Experiment/Experiment I (continued)

Study Name: 1179000
Experiment: 1179000 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 19/05/2008
Date Counted: 26/05/2008

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA pkm 101	WP2pKm 101
With Activation	DMSO		15 ± 1	17 ± 1	33 ± 4	140 ± 6	390 ± 11	297 ± 26
	Untreated		22 ± 1	19 ± 3	31 ± 10	145 ± 8	417 ± 24	305 ± 13
	SYN545682	3 µg	15 ± 5	17 ± 3	30 ± 4	131 ± 15	388 ± 18	286 ± 13
		10 µg	16 ± 2	16 ± 4	35 ± 6	138 ± 6	410 ± 3	256 ± 17
		33 µg	16 ± 4	14 ± 3	31 ± 4	143 ± 10	391 ± 17	241 ± 18
		100 µg	15 ± 1	15 ± 1	31 ± 2	156 ± 11	391 ± 27	262 ± 18
		333 µg	18 ± 5	16 ± 3	33 ± 9	131 ± 6	364 ± 6	259 ± 10
		1000 µg	14 ± 3 P M	12 ± 3 P M	29 ± 2 P M	129 ± 3 P M	365 ± 9 P M	248 ± 8 P M
		2500 µg	11 ± 1 P M	12 ± 3 P M	28 ± 1 P M	127 ± 6 P M	336 ± 13 P M	231 ± 9 P M
		5000 µg	14 ± 1 P M	13 ± 2 P M	20 ± 5 P M	112 ± 7 P M	336 ± 7 P M	218 ± 11 P M
	2-AA	2.5 µg	242 ± 12	139 ± 11	953 ± 65	1599 ± 94		
	2-AA	10.0 µg					1302 ± 107	1461 ± 21

Key to Positive Controls

2-AA	2-aminoanthracene

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 2. Summary of Results Experiment II

Study Name: 1179000 Study Code: RCC-CCR 1179000
Experiment: 1179000 HV2 Pre Date Plated: 20/05/2008
Assay Conditions: Date Counted: 27/05/2008

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA pkm 101	WP2pKm 101
Without Activation	DMSO		15 ± 4	9 ± 1	28 ± 5	108 ± 5	398 ± 19	272 ± 27
	Untreated		15 ± 4	11 ± 2	26 ± 3	129 ± 19	425 ± 37	313 ± 13
	SYN545682	3 µg	15 ± 6	9 ± 4	27 ± 4	114 ± 5	388 ± 10	268 ± 3
		10 µg	12 ± 3	12 ± 3	28 ± 14	106 ± 7	424 ± 20	259 ± 7
		33 µg	15 ± 2	7 ± 2	26 ± 5	112 ± 12	382 ± 20	254 ± 14
		100 µg	17 ± 4	9 ± 4	27 ± 7	111 ± 9	381 ± 21	281 ± 30
		333 µg	14 ± 2 P	10 ± 4 P	27 ± 7 P	114 ± 8 P	398 ± 29 P	239 ± 26 P
		1000 µg	12 ± 2 P M	7 ± 2 P M	22 ± 11 P M	104 ± 7 P M	365 ± 8 P M	229 ± 9 P M
		2500 µg	10 ± 4 P M	9 ± 2 P M	22 ± 3 P M	104 ± 6 P M	367 ± 9 P M	232 ± 3 P M
		5000 µg	11 ± 1 P M	8 ± 3 P M	19 ± 6 P M	107 ± 5 P M	348 ± 17 P M	249 ± 7 P M
	NaN3	10 µg	1962 ± 25			2047 ± 125		
	4-NOPD	10 µg			359 ± 13			
	4-NOPD	50 µg		116 ± 3				
	MMS	3.0 µL					2698 ± 230	3084 ± 288

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 2. Summary of Results Experiment II (continued)

Study Name: 1179000
Experiment: 1179000 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 20/05/2008
Date Counted: 27/05/2008

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA pkm 101	WP2pKm 101
With Activation	DMSO		19 ± 6	16 ± 1	40 ± 15	156 ± 14	456 ± 16	282 ± 22
	Untreated		16 ± 4	19 ± 2	43 ± 6	167 ± 19	471 ± 16	353 ± 3
	SYN545682	3 µg	16 ± 7	16 ± 4	34 ± 8	125 ± 9	435 ± 9	301 ± 22
		10 µg	18 ± 5	11 ± 6	39 ± 4	144 ± 6	434 ± 17	267 ± 22
		33 µg	23 ± 2	13 ± 1	37 ± 4	145 ± 28	442 ± 16	260 ± 37
		100 µg	21 ± 2	15 ± 2	35 ± 7	153 ± 23	454 ± 11	276 ± 36
		333 µg	19 ± 4	13 ± 4	38 ± 8	120 ± 20	408 ± 36	243 ± 33
		1000 µg	11 ± 1 P M	6 ± 2 P M	29 ± 2 P M	115 ± 9 P M	370 ± 11 P M	228 ± 7 P M
		2500 µg	11 ± 3 P M	9 ± 1 P M	31 ± 2 P M	111 ± 16 P M	348 ± 17 P M	237 ± 17 P M
		5000 µg	14 ± 6 P M	6 ± 5 P M	25 ± 2 P M	111 ± 6 P M	347 ± 18 P M	253 ± 24 P M
	2-AA	2.5 µg	259 ± 26	205 ± 25	1359 ± 154	2060 ± 103		
	2-AA	10.0 µg					1898 ± 313	640 ± 33

Key to Positive Controls

2-AA	2-aminoanthracene

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 3. Pre-Experiment and Experiment I: 1179000 VV Plate Incorporation

Study Name: 1179000
Experiment: 1179000 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 19/05/2008
Date Counted: 26/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**SYN545682**	3 µg	*15.7*	*4.0*	*1.0*	15, 20, 12
		10 µg	*17.0*	*3.0*	*1.1*	17, 20, 14
		33 µg	*10.7*	*2.9*	*0.7*	9, 9, 14
		100 µg	*14.7*	*5.1*	*1.0*	9, 19, 16
		333 µg	*17.0*	*7.2*	*1.1*	19, 23, 9
		1000 µg	*17.0*	*2.6*	*1.1*	20 P M, 15 P M, 16 P M
		2500 µg	*11.7*	*1.5*	*0.8*	13 P M, 12 P M, 10 P M
		5000 µg	*8.7*	*2.9*	*0.6*	7 P M, 12 P M, 7 P M
	DMSO		*15.0*	*5.0*		10, 20, 15
	Untreated Control		*17.0*	*2.6*		15, 20, 16
TA 1537	**SYN545682**	3 µg	*13.0*	*3.6*	*1.2*	10, 17, 12
		10 µg	*13.0*	*2.6*	*1.2*	14, 10, 15
		33 µg	*13.0*	*2.6*	*1.2*	14, 10, 15
		100 µg	*12.3*	*3.8*	*1.1*	8, 14, 15
		333 µg	*14.0*	*1.0*	*1.3*	14, 15, 13
		1000 µg	*10.7*	*3.1*	*1.0*	14 P M, 10 P M, 8 P M
		2500 µg	*10.0*	*3.0*	*0.9*	10 P M, 13 P M, 7 P M
		5000 µg	*10.3*	*2.5*	*0.9*	8 P M, 10 P M, 13 P M
	DMSO		*11.0*	*1.7*		12, 9, 12
	Untreated Control		*10.3*	*3.5*		7, 14, 10

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 3. Pre-Experiment and Experiment I: 1179000 VV Plate Incorporation (continued)

Study Name: 1179000
Experiment: 1179000 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 19/05/2008
Date Counted: 26/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 98	**SYN545682**	3 µg	*23.3*	*0.6*	*1.1*	23, 24, 23
		10 µg	*23.3*	*4.0*	*1.1*	27, 19, 24
		33 µg	*24.0*	*2.6*	*1.1*	27, 22, 23
		100 µg	*21.0*	*4.4*	*1.0*	23, 16, 24
		333 µg	*27.0*	*3.0*	*1.2*	27, 30, 24
		1000 µg	*21.7*	*3.2*	*1.0*	24 P M, 23 P M, 18 P M
		2500 µg	*17.0*	*3.0*	*0.8*	17 P M, 20 P M, 14 P M
		5000 µg	*16.7*	*1.5*	*0.8*	15 P M, 18 P M, 17 P M
	DMSO		*21.7*	*1.2*		21, 23, 21
	Untreated Control		*27.7*	*4.7*		26, 24, 33
TA 100	**SYN545682**	3 µg	*117.0*	*18.5*	*1.0*	116, 136, 99
		10 µg	*116.0*	*7.8*	*1.0*	120, 121, 107
		33 µg	*108.7*	*12.7*	*0.9*	116, 94, 116
		100 µg	*121.7*	*8.1*	*1.0*	129, 123, 113
		333 µg	*122.7*	*10.3*	*1.0*	120, 114, 134
		1000 µg	*112.0*	*9.5*	*0.9*	113 P M, 102 P M, 121 P M
		2500 µg	*111.3*	*9.3*	*0.9*	101 P M, 114 P M, 119 P M
		5000 µg	*102.7*	*5.0*	*0.9*	102 P M, 108 P M, 98 P M
	DMSO		*120.7*	*8.0*		113, 129, 120
	Untreated Control		*116.3*	*4.6*		111, 119, 119

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 3. Pre-Experiment and Experiment I: 1179000 VV Plate Incorporation (continued)

Study Name: 1179000
Experiment: 1179000 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 19/05/2008
Date Counted: 26/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA pkm 101	**SYN545682**	3 µg	*377.0*	*12.1*	*1.0*	384, 363, 384
		10 µg	*383.0*	*5.2*	*1.0*	386, 377, 386
		33 µg	*364.7*	*20.5*	*0.9*	344, 365, 385
		100 µg	*385.7*	*20.5*	*1.0*	362, 398, 397
		333 µg	*354.0*	*1.0*	*0.9*	354, 355, 353
		1000 µg	*355.3*	*14.0*	*0.9*	344 P M, 351 P M, 371 P M
		2500 µg	*352.3*	*10.3*	*0.9*	341 P M, 361 P M, 355 P M
		5000 µg	*346.7*	*23.6*	*0.9*	369 P M, 349 P M, 322 P M
	DMSO		*389.7*	*11.4*		393, 377, 399
	Untreated Control		*389.0*	*37.5*		372, 432, 363

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 3. Pre-Experiment and Experiment I: 1179000 VV Plate Incorporation (continued)

Study Name: 1179000
Experiment: 1179000 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 19/05/2008
Date Counted: 26/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2pKm 101	**SYN545682**	3 µg	*234.7*	*8.0*	*0.9*	234, 243, 227
		10 µg	*276.0*	*7.0*	*1.0*	269, 283, 276
		33 µg	*254.0*	*11.5*	*1.0*	255, 265, 242
		100 µg	*268.3*	*53.8*	*1.0*	262, 325, 218
		333 µg	*236.3*	*13.9*	*0.9*	248, 240, 221
		1000 µg	*238.0*	*9.0*	*0.9*	247 P M, 229 P M, 238 P M
		2500 µg	*239.0*	*11.8*	*0.9*	249 P M, 226 P M, 242 P M
		5000 µg	*229.3*	*9.1*	*0.9*	228 P M, 239 P M, 221 P M
	DMSO		*267.0*	*17.7*		251, 264, 286
	Untreated Control		*248.3*	*16.3*		230, 261, 254
TA 1535	**NaN3**	10 µg	*1875.0*	*89.2*	*125.0*	1789, 1869, 1967
TA 1537	**4-NOPD**	50 µg	*85.3*	*5.0*	*7.8*	86, 80, 90
TA 98	**4-NOPD**	10 µg	*390.3*	*8.7*	*18.0*	388, 400, 383
TA 100	**NaN3**	10 µg	*2211.7*	*86.6*	*18.3*	2113, 2247, 2275
WP2 uvrA pkm 101	**MMS**	3.0 µL	*4356.7*	*292.5*	*11.2*	4286, 4106, 4678
WP2pKm 101	**MMS**	3.0 µL	*3837.0*	*239.8*	*14.4*	3850, 4070, 3591

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 4. Pre-Experiment and Experiment I: 1179000 VV Plate Incorporation

Study Name: 1179000
Experiment: 1179000 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 19/05/2008
Date Counted: 26/05/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**SYN545682**	3 µg	*15.3*	*4.9*	*1.0*	12, 21, 13
		10 µg	*15.7*	*1.5*	*1.0*	17, 14, 16
		33 µg	*16.0*	*4.0*	*1.0*	12, 20, 16
		100 µg	*15.3*	*1.2*	*1.0*	16, 14, 16
		333 µg	*17.7*	*5.1*	*1.2*	22, 12, 19
		1000 µg	*14.0*	*2.6*	*0.9*	16 P M, 11 P M, 15 P M
		2500 µg	*11.0*	*1.0*	*0.7*	10 P M, 11 P M, 12 P M
		5000 µg	*14.3*	*0.6*	*0.9*	14 P M, 14 P M, 15 P M
	DMSO		*15.3*	*0.6*		15, 15, 16
	Untreated Control		*21.7*	*1.2*		21, 23, 21
TA 1537	**SYN545682**	3 µg	*17.3*	*2.5*	*1.0*	17, 20, 15
		10 µg	*15.7*	*3.5*	*0.9*	16, 19, 12
		33 µg	*13.7*	*3.2*	*0.8*	16, 10, 15
		100 µg	*15.0*	*1.0*	*0.9*	16, 15, 14
		333 µg	*15.7*	*3.1*	*0.9*	15, 19, 13
		1000 µg	*12.3*	*2.5*	*0.7*	12 P M, 10 P M, 15 P M
		2500 µg	*12.3*	*2.5*	*0.7*	10 P M, 12 P M, 15 P M
		5000 µg	*13.3*	*1.5*	*0.8*	12 P M, 15 P M, 13 P M
	DMSO		*16.7*	*0.6*		17, 16, 17
	Untreated Control		*19.3*	*2.5*		17, 19, 22

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 4. Pre-Experiment and Experiment I: 1179000 VV Plate Incorporation (continued)

Study Name: 1179000
Experiment: 1179000 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 19/05/2008
Date Counted: 26/05/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 98	**SYN545682**	3 µg	*30.3*	*3.5*	*0.9*	34, 27, 30
		10 µg	*35.0*	*6.0*	*1.0*	29, 41, 35
		33 µg	*31.0*	*3.6*	*0.9*	30, 35, 28
		100 µg	*31.3*	*1.5*	*0.9*	30, 31, 33
		333 µg	*33.0*	*8.5*	*1.0*	41, 34, 24
		1000 µg	*29.3*	*1.5*	*0.9*	28 P M, 29 P M, 31 P M
		2500 µg	*28.3*	*0.6*	*0.8*	28 P M, 29 P M, 28 P M
		5000 µg	*19.7*	*4.7*	*0.6*	25 P M, 18 P M, 16 P M
	DMSO		*33.3*	*3.8*		35, 29, 36
	Untreated Control		*31.3*	*9.5*		41, 22, 31
TA 100	**SYN545682**	3 µg	*131.0*	*15.4*	*0.9*	118, 148, 127
		10 µg	*138.0*	*6.1*	*1.0*	134, 145, 135
		33 µg	*142.7*	*10.3*	*1.0*	140, 134, 154
		100 µg	*156.3*	*11.2*	*1.1*	152, 148, 169
		333 µg	*131.3*	*5.9*	*0.9*	129, 138, 127
		1000 µg	*129.0*	*3.0*	*0.9*	129 P M, 132 P M, 126 P M
		2500 µg	*127.0*	*6.0*	*0.9*	133 P M, 121 P M, 127 P M
		5000 µg	*112.0*	*7.0*	*0.8*	109 P M, 120 P M, 107 P M
	DMSO		*140.0*	*6.2*		142, 133, 145
	Untreated Control		*145.3*	*7.5*		141, 154, 141

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 4. Pre-Experiment and Experiment I: 1179000 VV Plate Incorporation (continued)

Study Name: 1179000
Experiment: 1179000 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 19/05/2008
Date Counted: 26/05/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA pkm 101	**SYN545682**	3 µg	*388.3*	*17.6*	*1.0*	399, 368, 398
		10 µg	*410.0*	*2.6*	*1.1*	407, 412, 411
		33 µg	*391.0*	*16.6*	*1.0*	384, 410, 379
		100 µg	*391.3*	*27.1*	*1.0*	385, 421, 368
		333 µg	*363.7*	*5.5*	*0.9*	361, 360, 370
		1000 µg	*364.7*	*8.5*	*0.9*	355 P M, 371 P M, 368 P M
		2500 µg	*336.0*	*12.5*	*0.9*	349 P M, 335 P M, 324 P M
		5000 µg	*336.3*	*7.0*	*0.9*	343 P M, 329 P M, 337 P M
	DMSO		*390.3*	*10.6*		392, 379, 400
	Untreated Control		*416.7*	*24.3*		395, 412, 443

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 4. Pre-Experiment and Experiment I: 1179000 VV Plate Incorporation (continued)

Study Name: 1179000
Experiment: 1179000 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 19/05/2008
Date Counted: 26/05/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2pKm 101	**SYN545682**	3 µg	*285.7*	*13.3*	*1.0*	297, 271, 289
		10 µg	*256.0*	*16.6*	*0.9*	268, 263, 237
		33 µg	*241.3*	*17.6*	*0.8*	260, 239, 225
		100 µg	*261.7*	*17.5*	*0.9*	262, 279, 244
		333 µg	*258.7*	*10.1*	*0.9*	264, 247, 265
		1000 µg	*248.3*	*8.1*	*0.8*	257 P M, 247 P M, 241 P M
		2500 µg	*231.0*	*9.2*	*0.8*	221 P M, 239 P M, 233 P M
		5000 µg	*218.0*	*10.5*	*0.7*	217 P M, 229 P M, 208 P M
	DMSO		*297.0*	*25.6*		324, 273, 294
	Untreated Control		*304.7*	*12.5*		319, 296, 299
TA 1535	**2-AA**	2.5 µg	*241.7*	*11.5*	*15.8*	230, 242, 253
TA 1537	**2-AA**	2.5 µg	*139.0*	*11.1*	*8.3*	151, 137, 129
TA 98	**2-AA**	2.5 µg	*952.7*	*64.5*	*28.6*	953, 888, 1017
TA 100	**2-AA**	2.5 µg	*1599.0*	*94.2*	*11.4*	1494, 1627, 1676
WP2 uvrA pkm 101	**2-AA**	10.0 µg	*1302.3*	*106.6*	*3.3*	1221, 1263, 1423
WP2pKm 101	**2-AA**	10.0 µg	*1461.3*	*20.8*	*4.9*	1478, 1438, 1468

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate
M Manual count

TABLE 5. Experiment II: 1179000 HV2 Pre-Incubation

Study Name: 1179000
Experiment: 1179000 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 20/05/2008
Date Counted: 27/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**SYN545682**	3 µg	*15.0*	*5.6*	*1.0*	14, 10, 21
		10 µg	*12.0*	*3.0*	*0.8*	15, 12, 9
		33 µg	*15.3*	*1.5*	*1.0*	15, 14, 17
		100 µg	*17.0*	*3.6*	*1.1*	21, 14, 16
		333 µg	*14.0*	*1.7*	*0.9*	13 P, 13 P, 16 P
		1000 µg	*12.3*	*2.1*	*0.8*	14 P M, 10 P M, 13 P M
		2500 µg	*10.0*	*4.4*	*0.7*	7 P M, 8 P M, 15 P M
		5000 µg	*11.3*	*0.6*	*0.8*	11 P M, 11 P M, 12 P M
	DMSO		*15.0*	*3.6*		12, 14, 19
	Untreated Control		*15.0*	*3.6*		14, 19, 12
TA 1537	**SYN545682**	3 µg	*8.7*	*4.0*	*1.0*	5, 13, 8
		10 µg	*12.3*	*2.9*	*1.4*	14, 14, 9
		33 µg	*7.3*	*2.1*	*0.8*	8, 9, 5
		100 µg	*9.3*	*3.5*	*1.1*	6, 9, 13
		333 µg	*10.3*	*4.2*	*1.2*	7 P, 9 P, 15 P
		1000 µg	*7.3*	*2.1*	*0.8*	5 P M, 8 P M, 9 P M
		2500 µg	*9.3*	*1.5*	*1.1*	8 P M, 11 P M, 9 P M
		5000 µg	*7.7*	*3.1*	*0.9*	5 P M, 11 P M, 7 P M
	DMSO		*8.7*	*0.6*		9, 8, 9
	Untreated Control		*11.3*	*2.1*		13, 12, 9

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 5 Experiment II: 1179000 HV2 Pre-Incubation (continued)

Study Name: 1179000
Experiment: 1179000 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 20/05/2008
Date Counted: 27/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 98	**SYN545682**	3 µg	*27.3*	*3.5*	*1.0*	31, 27, 24
		10 µg	*28.3*	*14.5*	*1.0*	45, 21, 19
		33 µg	*25.7*	*4.9*	*0.9*	28, 29, 20
		100 µg	*27.3*	*7.4*	*1.0*	33, 19, 30
		333 µg	*26.7*	*7.2*	*1.0*	23 P, 35 P, 22 P
		1000 µg	*22.0*	*10.6*	*0.8*	30 P M, 10 P M, 26 P M
		2500 µg	*21.7*	*3.1*	*0.8*	25 P M, 21 P M, 19 P M
		5000 µg	*19.0*	*5.6*	*0.7*	13 P M, 20 P M, 24 P M
	DMSO		*27.7*	*5.0*		33, 27, 23
	Untreated Control		*26.3*	*2.9*		28, 23, 28
TA 100	**SYN545682**	3 µg	*114.3*	*5.0*	*1.1*	109, 115, 119
		10 µg	*106.0*	*6.9*	*1.0*	102, 114, 102
		33 µg	*112.0*	*11.8*	*1.0*	99, 115, 122
		100 µg	*110.7*	*9.1*	*1.0*	107, 104, 121
		333 µg	*114.3*	*8.0*	*1.1*	122 P, 115 P, 106 P
		1000 µg	*104.3*	*6.7*	*1.0*	112 P M, 100 P M, 101 P M
		2500 µg	*104.0*	*6.2*	*1.0*	99 P M, 111 P M, 102 P M
		5000 µg	*107.0*	*5.3*	*1.0*	101 P M, 109 P M, 111 P M
	DMSO		*108.0*	*5.2*		111, 111, 102
	Untreated Control		*129.3*	*19.3*		151, 114, 123

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 5 Experiment II: 1179000 HV2 Pre-Incubation (continued)

Study Name: 1179000
Experiment: 1179000 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 20/05/2008
Date Counted: 27/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA pkm 101	**SYN545682**	3 µg	*388.0*	*10.4*	*1.0*	395, 376, 393
		10 µg	*424.3*	*19.6*	*1.1*	413, 447, 413
		33 µg	*382.3*	*19.7*	*1.0*	397, 390, 360
		100 µg	*381.0*	*20.7*	*1.0*	375, 404, 364
		333 µg	*398.0*	*28.6*	*1.0*	431 P, 381 P, 382 P
		1000 µg	*365.3*	*8.1*	*0.9*	356 P M, 371 P M, 369 P M
		2500 µg	*366.7*	*9.3*	*0.9*	377 P M, 364 P M, 359 P M
		5000 µg	*348.3*	*17.0*	*0.9*	361 P M, 329 P M, 355 P M
	DMSO		*397.7*	*18.5*		419, 388, 386
	Untreated Control		*424.7*	*36.6*		390, 421, 463

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 5 Experiment II: 1179000 HV2 Pre-Incubation (continued)

Study Name: 1179000
Experiment: 1179000 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 20/05/2008
Date Counted: 27/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2pKm 101	**SYN545682**	3 µg	*268.0*	*3.0*	*1.0*	268, 271, 265
		10 µg	*259.0*	*7.2*	*1.0*	267, 253, 257
		33 µg	*253.7*	*13.9*	*0.9*	242, 250, 269
		100 µg	*281.0*	*30.4*	*1.0*	308, 248, 287
		333 µg	*238.7*	*26.3*	*0.9*	267 P, 234 P, 215 P
		1000 µg	*229.3*	*8.6*	*0.8*	220 P M, 237 P M, 231 P M
		2500 µg	*231.7*	*3.1*	*0.9*	231 P M, 235 P M, 229 P M
		5000 µg	*248.7*	*7.0*	*0.9*	248 P M, 256 P M, 242 P M
	DMSO		*272.3*	*27.2*		301, 269, 247
	Untreated Control		*312.7*	*13.0*		326, 312, 300
TA 1535	**NaN3**	10 µg	*1962.3*	*25.4*	*130.8*	1933, 1978, 1976
TA 1537	**4-NOPD**	50 µg	*115.7*	*2.5*	*13.3*	116, 113, 118
TA 98	**4-NOPD**	10 µg	*358.7*	*12.6*	*13.0*	372, 357, 347
TA 100	**NaN3**	10 µg	*2046.7*	*125.1*	*19.0*	2183, 2020, 1937
WP2 uvrA pkm 101	**MMS**	3.0 µL	*2698.3*	*229.9*	*6.8*	2447, 2898, 2750
WP2pKm 101	**MMS**	3.0 µL	*3084.3*	*287.6*	*11.3*	2816, 3049, 3388

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 6. Experiment II: 1179000 HV2 Pre-Incubation

Study Name: 1179000
Experiment: 1179000 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 20/05/2008
Date Counted: 27/05/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**SYN545682**	3 µg	*16.3*	*6.5*	*0.9*	16, 10, 23
		10 µg	*18.3*	*4.7*	*1.0*	20, 22, 13
		33 µg	*23.3*	*2.3*	*1.2*	22, 22, 26
		100 µg	*21.3*	*2.1*	*1.1*	23, 22, 19
		333 µg	*19.0*	*4.4*	*1.0*	16, 24, 17
		1000 µg	*11.0*	*1.0*	*0.6*	11 P M, 10 P M, 12 P M
		2500 µg	*11.3*	*3.2*	*0.6*	10 P M, 9 P M, 15 P M
		5000 µg	*14.3*	*6.0*	*0.8*	15 P M, 20 P M, 8 P M
	DMSO		*18.7*	*6.1*		20, 12, 24
	Untreated Control		*15.7*	*3.8*		20, 14, 13
TA 1537	**SYN545682**	3 µg	*16.3*	*4.2*	*1.0*	15, 13, 21
		10 µg	*11.3*	*5.5*	*0.7*	5, 14, 15
		33 µg	*13.3*	*1.2*	*0.8*	14, 12, 14
		100 µg	*14.7*	*2.1*	*0.9*	13, 17, 14
		333 µg	*13.0*	*4.4*	*0.8*	16, 15, 8
		1000 µg	*6.3*	*2.3*	*0.4*	5 P M, 5 P M, 9 P M
		2500 µg	*9.3*	*1.2*	*0.6*	10 P M, 8 P M, 10 P M
		5000 µg	*6.3*	*4.7*	*0.4*	8 P M, 1 P M, 10 P M
	DMSO		*16.3*	*0.6*		17, 16, 16
	Untreated Control		*19.0*	*2.0*		21, 17, 19

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 6 Experiment II: 1179000 HV2 Pre-Incubation (continued)

Study Name: 1179000
Experiment: 1179000 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 20/05/2008
Date Counted: 27/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 98	**SYN545682**	3 µg	*34.0*	*7.5*	*0.9*	42, 33, 27
		10 µg	*39.3*	*4.0*	*1.0*	37, 44, 37
		33 µg	*36.7*	*3.5*	*0.9*	37, 40, 33
		100 µg	*34.7*	*7.2*	*0.9*	31, 30, 43
		333 µg	*37.7*	*8.3*	*0.9*	47, 35, 31
		1000 µg	*29.3*	*1.5*	*0.7*	29 P M, 31 P M, 28 P M
		2500 µg	*31.3*	*2.1*	*0.8*	32 P M, 29 P M, 33 P M
		5000 µg	*24.7*	*2.1*	*0.6*	23 P M, 27 P M, 24 P M
	DMSO		*40.0*	*15.1*		26, 56, 38
	Untreated Control		*42.7*	*6.0*		37, 42, 49
TA 100	**SYN545682**	3 µg	*125.0*	*8.9*	*0.8*	135, 118, 122
		10 µg	*144.0*	*5.6*	*0.9*	145, 149, 138
		33 µg	*145.0*	*27.6*	*0.9*	136, 123, 176
		100 µg	*153.3*	*23.2*	*1.0*	180, 138, 142
		333 µg	*120.3*	*19.6*	*0.8*	115, 142, 104
		1000 µg	*115.3*	*8.5*	*0.7*	112 P M, 125 P M, 109 P M
		2500 µg	*111.3*	*15.9*	*0.7*	98 P M, 129 P M, 107 P M
		5000 µg	*111.0*	*6.0*	*0.7*	117 P M, 111 P M, 105 P M
	DMSO		*156.0*	*13.5*		143, 155, 170
	Untreated Control		*167.0*	*19.1*		155, 189, 157

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 6 Experiment II: 1179000 HV2 Pre-Incubation (continued)

Study Name: 1179000
Experiment: 1179000 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 20/05/2008
Date Counted: 27/05/2008

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA pkm 101	**SYN545682**	3 µg	*435.3*	*9.1*	*1.0*	439, 442, 425
		10 µg	*434.0*	*16.6*	*1.0*	446, 415, 441
		33 µg	*442.3*	*16.3*	*1.0*	428, 460, 439
		100 µg	*453.7*	*11.1*	*1.0*	442, 455, 464
		333 µg	*408.3*	*35.8*	*0.9*	429, 429, 367
		1000 µg	*370.0*	*10.5*	*0.8*	371 P M, 359 P M, 380 P M
		2500 µg	*348.3*	*17.0*	*0.8*	361 P M, 329 P M, 355 P M
		5000 µg	*346.7*	*17.6*	*0.8*	361 P M, 327 P M, 352 P M
	DMSO		*455.7*	*15.9*		474, 446, 447
	Untreated Control		*470.7*	*16.2*		488, 468, 456

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 6 Experiment II: 1179000 HV2 Pre-Incubation (continued)

Study Name: 1179000
Experiment: 1179000 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1179000
Date Plated: 20/05/2008
Date Counted: 27/05/2008

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2pKm 101	**SYN545682**	3 µg	*301.0*	*21.7*	*1.1*	290, 326, 287
		10 µg	*267.0*	*21.7*	*0.9*	290, 264, 247
		33 µg	*260.0*	*36.7*	*0.9*	268, 220, 292
		100 µg	*276.0*	*36.0*	*1.0*	276, 240, 312
		333 µg	*243.3*	*33.2*	*0.9*	263, 262, 205
		1000 µg	*227.7*	*7.0*	*0.8*	227 P M, 221 P M, 235 P M
		2500 µg	*237.0*	*16.8*	*0.8*	224 P M, 256 P M, 231 P M
		5000 µg	*253.3*	*23.6*	*0.9*	251 P M, 278 P M, 231 P M
	DMSO		*281.7*	*22.3*		273, 307, 265
	Untreated Control		*353.0*	*2.6*		350, 355, 354
TA 1535	**2-AA**	2.5 µg	*259.0*	*25.7*	*13.9*	279, 268, 230
TA 1537	**2-AA**	2.5 µg	*205.3*	*25.1*	*12.6*	229, 208, 179
TA 98	**2-AA**	2.5 µg	*1358.7*	*154.1*	*34.0*	1404, 1187, 1485
TA 100	**2-AA**	2.5 µg	*2060.3*	*103.2*	*13.2*	1975, 2175, 2031
WP2 uvrA pkm 101	**2-AA**	10.0 µg	*1897.7*	*312.6*	*4.2*	1547, 1999, 2147
WP2pKm 101	**2-AA**	10.0 µg	*561.0*	*104.8*	*2.0*	440, 619, 624

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate
M Manual count

APPENDICES SECTION

APPENDIX 1 Historical Control Data

These data represent the laboratory´s historical control data from January 2007 until July 2007 representing approx. 200 experiments (WP2 uvrA pKM101 the historical data from January 2007 until November 2007 are based on approx. 100 experiments; the control data for strain WP2 pKM101 are from January 2007 until November 2007 are based on approx. 20 experiments.

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	17	4.51	7	40	21	4.95	8	41
	Negative control	17	4.57	9	34	21	5.55	9	42
	Positive control	1878	203.83	852	2347	270	83.95	98	636
TA1537	Solvent control	11	2.91	6	23	16	4.19	6	35
	Negative control	11	2.97	6	24	17	4.97	7	37
	Positive control	125	41.01	75	424	180	64.61	73	475
TA 98	Solvent control	32	6.78	18	66	40	6.24	24	64
	Negative control	35	6.67	17	62	41	6.56	23	67
	Positive control	534	163.95	172	1916	1193	491.42	184	2759
TA 100	Solvent control	138	25.59	84	213	157	27.89	94	254
	Negative control	145	21.66	97	210	161	25.76	94	217
	Positive control	1953	492.35	572	2943	1763	713.99	542	3886
WP2 uvrA pKM101	Solvent control	251	74.68	121	442	287	87.92	117	484
	Negative control	272	63.90	164	457	302	92.02	129	529
	Positive control	2810	1291.6	852	5238	1956	494.6	869	3999
WP2 pKM101	Solvent control	142	75.7	57	276	159	88.02	50	326
	Negative control	157	68.06	93	267	172	76.64	77	313
	Positive control	2923	1010.3	1176	4774	1565	1126.2	406	3700

Mean = mean value of revertants/plate
SD = standard deviation Min = minimal value/Max = maximal value

APPENDIX 2 Copy of GLP Certificate

HESSEN

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

RCC – Cytotest Cell Research GmbH
RCC – Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Rossdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
6 Prüfungen zur Bestimmung von Rückständen
8 Analytische Prüfungen an biologischen Materialien
9 Virussicherheitsprüfungen

2 Toxicity studies
3 Mutagenicity studies
6 Residues
8 Analytical studies on biological materials
9 Virus validation studies

02.09.2006
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP- Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

Th. Zimmermann, Referent, Wiesbaden, den **19. Januar 2007**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, LÄNDLICHEN RAUM UND VERBRAUCHERSCHUTZ

Hess. Ministerium für Umwelt, ländlichen Raum und Verbraucherschutz,
Mainzer Straße 80 D65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority

APPENDIX 3 Certificate of Analysis

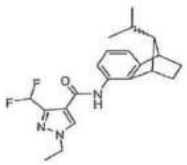
syngenta

GLP Testing Facility WMU
Analytical Development &
Product Chemistry GS2131

Syngenta Crop Protection
Münchwilen AG
Breitenloh 5
CH-4333 Münchwilen

Certificate of Analysis

SYN545682



BPS 1266/1 - Purity 98.4 %

Batch Identification	**BPS 1266/1**
Product Code	**SYN545682**
Other Product Code(s)	---
CA Reg. No.	---
CA Index Name	---
IUPAC Name	3-difluoromethyl-1-ethyl-1H-pyrazole-4-carboxylic acid (9-isopropyl-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide
Molecular formula	$C_{21}H_{25}F_2N_3O$
Molecular mass	373.5
Chemical Analysis	
– **Identity ***	**confirmed**
– **Content of SYN545682 * (sum of epimers)**	**98.4 % (88.2 % syn-epimer, 10.2 % anti-epimer)**
Methodology used for Characterization	Cap. GC, NMR (qualitative and quantitative)
Physical Analysis	
– **Appearance ***	**off-white solid**
Stability:	
– **Storage Temperature**	**< 30°C**
– **Reanalysis Date**	**End of April 2010**

The stability of this test substance will be controlled by reanalysis of material held in the inventory at Syngenta Crop Protection Münchwilen AG at the appropriate time.

This Certificate of Analysis is summarizing data which originate either from a single study or from several individual studies which have been performed in compliance with GLP. Tests marked with an asterisk (*) have been conducted within a single study/as individual studies. Raw data, documentation, study plans, any amendments to study plans and reports pertaining to this/these studie(s) are stored under the study number(s) referenced below within the archives of the GLP Testing Facility WMU at Syngenta Crop Protection Münchwilen AG.

Characterization: 118523

Authorisation:

22-Apr-2008

Dr. R. Kettner
Analytical Development & Product Chemistry

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