harlan®

Azoxystrobin/SYN545192

Azoxystrobin/SYN545192 EC (A17961A) - Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay

Final Report

DATA REQUIREMENT(S):	OECD 471 (1997) EPA OPPTS 870.5100 (1998) EC 440/2008 B.13/14 (2008)
AUTHOR(S):	Dipl. Biol. Andrea Sokolowski
STUDY COMPLETION DATE:	17 June 2011
PERFORMING LABORATORY:	Harlan Cytotest Cell Research GmbH (Harlan CCR) In den Leppsteinswiesen 19 64380 Rossdorf, Germany
LABORATORY PROJECT ID:	Report Number: 1402001 Study Number: 1402001 Task Number: TK0057404
SPONSOR(S):	Syngenta Ltd Jealott's Hill International Research Centre Bracknell, Berkshire RG42 6EY, United Kingdom

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

This page intentionally left blank.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study performed in the test facility of Harlan CCR, 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), in its currently valid version

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

These procedures are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHW, MAFF, and METI).

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

Performing Laboratory
Harlan
Cytotest Cell Research GmbH (Harlan CCR)
In den Leppsteinswiesen 19
64380 Rossdorf, Germany

Dipl. Biol. Andrea Sokolowski
Study Director Bacterial Systems

.....
Date: 17 June 2011

FLAGGING STATEMENT

This page intentionally left blank.

QUALITY ASSURANCE STATEMENT

Study Number: 1402001

Test Item: Azoxystrobin/SYN545192 EC (A17961A)

Study Director: Dipl. Biol. Andrea Sokolowski

Title: Azoxystrobin/SYN545192 EC (A17961A)-
Salmonella Typhimurium and
Escherichia Coli Reverse Mutation Assay

The general facilities and activities of Harlan CCR 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:	03 March 2011	03 March 2011
Process Inspection Preparation for Application and Application:	24 March 2011	24 March 2011
Draft Report:	23 May 2011	23 May 2011

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

Head of Quality Assurance Unit Frauke Hermann

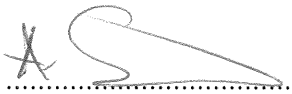
S. Ebert Sabine Ebert

.....

Date: 17 June 2011

PROJECT STAFF SIGNATURES

Study Director — Dipl. Biol. Andrea Sokolowski



.....

Date: 17 June 2011

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:	Harlan Cytotest Cell Research GmbH (Harlan CCR) In den Leppsteinswiesen 19 64380 Rossdorf Germany
Contracting Institute:	Harlan Laboratories Ltd. 4452 Itingen Switzerland
Reference Number:	D23642

Study dates

Study initiation date:	07 March 2011
Experimental start date:	30 March 2011
Experimental termination date:	19 April 2011

Deviations from the guidelines

None

Retention of samples

Raw data and a sample of the test item.

Performing laboratory test item reference number

S 12187 11

Other

Harlan CCR will archive:

Raw data, study plan, report, and specimens (if any) for at least 3 years at the test facility's archive. Thereafter, the material will be transferred to the GLP archive of Harlan Laboratories Ltd. in Füllinsdorf, Switzerland for archiving the remaining time up to a total archiving period of 15 years. No data will be discarded without the sponsor's written consent.

A sample of the test item will be archived two years after the expiration date provided by the sponsor. If no expiration date is given, the archiving period will be the required 15 years. Thereafter the samples will be discarded without further notice.

Good laboratory practice

The study was performed in compliance with:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1), in its currently valid version

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

These procedures are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHW, MAFF, and METI).

Deviations to study plan

None

Distribution of the report

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

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

1.1 Study Design

This study was performed to investigate the potential of azoxystrobin/SYN545192 EC (A17961A) to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, and the *Escherichia coli* strains WP2 *uvrA* pKM101 and WP2 pKM101.

1.2 Results

Reduced background growth was observed at higher concentrations in strains TA1535, TA1537, TA98 without S9 mix and in strain TA 100 with and without S9 mix in experiment I: In experiment II, reduced background growth was observed at higher concentrations in all strains used.

Toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), were observed at higher concentrations in all strains without S9 mix and in strains TA100 and WP2 pKM101 with S9 mix in experiment I, and with and without S9 mix in all strains in experiment II.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with azoxystrobin/SYN545192 EC (A17961A) 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, azoxystrobin/SYN545192 EC (A17961A) 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 azoxystrobin/SYN545192 EC (A17961A) to induce gene mutations by means of the Salmonella typhimurium and Escherichia coli reverse mutation assay. 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 (1). 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 assure reliable detection of mutagens that may be specific to one tester strain or locus. 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 (TA1535, TA100, WP2 *uvrA* pKM101, and WP2 pKM101) and frameshift (TA1537, TA98) 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

"United States Environmental Protection Agency, Health Effects Test Guideline OPPTS 870.5100 (1998). Bacterial Reverse Mutation Test."

"Commission Regulation (EC) No. 440/2008 B13/14", dated May 30, 2008

3.0 MATERIALS AND METHODS

3.1 Test Item

Internal Test Item Number: S12187 11

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

Identity:	Azoxystrobin/SYN545192 EC (100/050)
Product Code:	A17961A
Batch No.:	SMU0GL002
Purity:	Not indicated by the sponsor
Active Ingredient Content:	Azoxystrobin (ICI15505) 99.9 g/L corresponding to 9.48 % w/w SYN545192 50.3 g/L corresponding to 4.77 % w/w
Stability in Solvent:	Not indicated by the sponsor
Storage:	At room temperature < 30°C
Reanalysis Date:	End of July 2013

On the day of the experiment, the test item azoxystrobin/SYN545192 EC (A17961A) was dissolved in DMSO (MERCK, D-64293 Darmstadt; purity > 99 %). The solvent was chosen because of its solubilisation properties and its relative non-toxicity to the bacteria (2).

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:	TA1535, TA100
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:	TA1537, TA98
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* pKM101, WP2 pKM101
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

With metabolic activation

Strains:	TA1535, TA1537, TA98, TA100, WP2 *uvrA* pKM101, WP2 pKM101
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 (TA1535, TA1537, TA98, TA100), 10 µg/plate (WP2 *uvrA* pKM101, WP2 pKM101)

The stability of the positive control substances in solution was not determined 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 pKM101 carries the ampicillin resistance marker (3).

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* strains 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**
TA1537	*his* C 3076; *rfa*$^-$; *uvrB*$^-$	frame shift mutations
TA98	*his* D 3052; *rfa*$^-$; *uvrB*$^-$; R-factor	" "
TA1535	*his* G 46; *rfa*$^-$; *uvrB*$^-$	base-pair substitutions
TA100	*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 by Harlan CCR according to B. Ames et al. (5) and D. Maron and B. Ames (3). In this way it is ensured that the experimental conditions set down by Ames are fulfilled.

The bacterial strains TA1535, TA1537, TA98, TA100, WP2 *uvrA* pKM101, and WP2pKM101 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 stock cultures 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* pKM101, and WP2 pKM101. This nutrient medium contains per litre:

8 g 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. The optical density of the bacteria was determined by absorption measurement and the obtained values indicated that the bacteria were harvested at the late exponential or early stationary phase (10^8-10^9 cells/mL).

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*:
7.0 g Agar Agar*	7.0 g Agar Agar*
6.0 g NaCl*	6.0 g NaCl*
10.5mg L-Histidine×HCl×H_2O*	10.2 mg Tryptophan*
12.2mg 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 the form of mammalian microsome enzyme activation mixture.

3.4.1 S9 (Preparation by Harlan CCR)

Phenobarbital/ß-naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 – 12 weeks old male Wistar rats (Hsd Cpb: WU; weight approx. 220 – 320 g, Harlan Laboratories B. V., 5960 AD Horst, The Netherlands) induced by intraperitoneal administrations of 80 mg/kg b.w. phenobarbital (Desitin; 22335 Hamburg, Germany) and by peroral administrations of ß-naphthoflavone (Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) 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+3 parts) 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 27.2 mg/mL (lot no. R081010) in experiment I, and 30.7 (lot no. R060111) in experiment II.

3.4.2 S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor 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
4	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.*(5).

3.5 Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with all strains. Eight concentrations were tested for toxicity and mutation induction each with three replicate plates. The experimental conditions in this pre-experiment were the same as described below for experiment I (plate incorporation test).

Toxicity of the test item results in a reduction in the number of spontaneous revertants (below a factor of 0.5) or a clearing of the bacterial background lawn.

The pre-experiment may be reported as the main experiment I if the acceptance criteria are met (see section 3.8.2), otherwise it will be reported as the pre-experiment:

3.6 Dose Selection

In the pre-experiment the concentration range of the test item was 3 - 5000 µg/plate. Since the criteria mentioned above were met, the pre-experiment is reported as main experiment. The following concentrations were tested in experiment II:

Without metabolic activation: 1; 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
With metabolic activation: 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

For the pre-incubation method 100 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer* and 100 µL bacteria suspension were mixed in a test tube and incubated 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 (6).

* Substitution buffer: 7 parts of the 100 mM sodium-ortho-phosphate-buffer pH 7.4 with 3 parts of KCl solution 0.15 M

3.8 Data Evaluation

3.8.1 Data recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, Suffolk CB9 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
- A minimum of five analysable dose levels should be present with at least four dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5.

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 (1).

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration (6).

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. Since the criteria mentioned above were met, the pre-experiment is reported as main experiment. The following concentrations were tested in experiment II:

Without metabolic activation: 1; 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
With metabolic activation: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

4.2 Discussion

The test item azoxystrobin/SYN545192 EC (A17961A) 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 TA1535, TA1537, TA98, and TA100, and the *Escherichia coli* strains WP2 *uvrA* pKM101 and WP2 pKM101.

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

Pre-Experiment/Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:
Without metabolic activation: 1; 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
With metabolic activation: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Reduced background growth was observed at the following concentrations (µg/plate):

Strain	Experiment I		Experiment II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA 1535	1000 - 5000	/	1000 - 5000	2500, 5000
TA 1537	1000 - 5000	/	333 - 5000	1000 - 5000
TA 98	2500, 5000	/	2500, 5000	2500, 5000
TA 100	1000 - 5000	2500, 5000	333 - 5000	2500, 5000
WP2 pKM101	/	/	5000	/
WP2 uvrA pKM101	/	/	5000	/

/ no reduced background growth observed

Toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), were observed at the following concentrations (µg/plate):

Strain	Experiment I		Experiment II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA 1535	2500, 5000	/	1000 - 5000	2500, 5000
TA 1537	1000 - 5000	/	1000 - 5000	2500, 5000
TA 98	5000	/	2500, 5000	5000
TA 100	333 - 5000	5000	333 - 5000	2500, 5000
WP2 pKM101	5000	5000	2500, 5000	5000
WP2 uvrA pKM101	5000	/	5000	5000

/ no toxic effects observed

Precipitation of the test item was observed in the overlay agar from 1000 - 5000 µg/ plate in the test tubes and on the incubated agar plates in both experiments. The undissolved particles had no influence on the data recording.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with azoxystrobin/SYN545192 EC (A17961A)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.

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

The laboratory´s historical control range was exceeded in the untreated and solvent control of strain WP2 pKM101 with and without metabolic activation in experiment I, and without metabolic activation in experiment II. These deviations are judged to be based on biologically irrelevant fluctuations in the number of colonies and have no impact on the outcome of the study.

5.0 CONCLUSIONS

During the described mutagenicity tests and under the experimental conditions reported, azoxystrobin/SYN545192 EC (A17961A) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Azoxystrobin/SYN545192 EC (A17961A) is considered to be non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

6.0 REFERENCES

1. Hollstein,M., J. McCann, F.A. Angelosanto, and W.W. Nichols (1979)
   Short-term tests for carcinogens and mutagens
   Mutation Res. 65, 133-226

2. Maron D.M., J. Katzenellenbogen, and B.N. Ames (1981)
   Compatibility of organic solvents with the Salmonella/Microsome Test
   Mutation Res. 88, 343-350

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

4. Green, M.H.L. and W.J. Muriel (1976)
   Mutagen Testing Using TRP^+ Reversion in Escherichia Coli
   Mutation. Res. 38, 3- 32

5. Ames, B.N., J. McCann, and E. Yamasaki (1977)
   Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test
   In: B.J. Kilbey et al. (Eds.)"Handbook of Mutagenicity Test Procedures" Elsevier, Amsterdam, 1-17

6. de Serres F.J. and M.D. Shelby (1979)
   Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay
   Mutation Res. 64, 159-165

TABLES SECTION

TABLE 1 Summary of Results Pre-Experiment/Experiment I

Study Name: 1402001
Experiment: 1402001 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 30/03/2011
Date Counted: 04/04/2011

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	DMSO		13 ± 2	10 ± 3	28 ± 7	172 ± 9	230 ± 3	460 ± 53
	Untreated		16 ± 3	11 ± 2	34 ± 5	181 ± 7	294 ± 12	438 ± 9
	Azoxystrobin/	3 µg	14 ± 5	9 ± 3	26 ± 5	159 ± 7	247 ± 23	426 ± 18
	SYN545192 EC)	10 µg	15 ± 2	11 ± 2	32 ± 5	180 ± 14	241 ± 14	424 ± 16
	(A17961A)	33 µg	14 ± 2	13 ± 1	26 ± 4	161 ± 27	253 ± 8	444 ± 16
		100 µg	11 ± 1	9 ± 3	30 ± 4	148 ± 12	245 ± 22	416 ± 26
		333 µg	14 ± 2	7 ± 2	26 ± 8	77 ± 13	211 ± 19	416 ± 7
		1000 µg	6 ± 2 P R M	3 ± 1 P M R	16 ± 3 P	67 ± 6 P R	195 ± 5 P	376 ± 9 P
		2500 µg	4 ± 1 P M R	2 ± 1 P M R	13 ± 2 P R	33 ± 4 P M R	161 ± 7 P	306 ± 31 P
		5000 µg	3 ± 1 P M R	1 ± 1 P M R	8 ± 2 P M R	12 ± 3 P M R	91 ± 18 P	147 ± 17 P
	NaN3	10 µg	2238 ± 14			2264 ± 35		
	4-NOPD	10 µg			370 ± 2			
	4-NOPD	50 µg		69 ± 9				
	MMS	3.0 µL					4109 ± 14	3862 ± 93
With Activation	DMSO		19 ± 2	11 ± 3	32 ± 4	168 ± 16	278 ± 25	505 ± 17
	Untreated		21 ± 5	14 ± 4	47 ± 2	183 ± 22	309 ± 22	495 ± 20
	Azoxystrobin/	3 µg	20 ± 4	12 ± 4	33 ± 1	168 ± 7	282 ± 19	488 ± 22
	SYN545192 EC)	10 µg	20 ± 8	10 ± 4	37 ± 3	170 ± 22	285 ± 13	470 ± 32
	(A17961A)	33 µg	17 ± 4	12 ± 5	40 ± 2	170 ± 9	276 ± 9	487 ± 13
		100 µg	17 ± 5	10 ± 6	37 ± 4	165 ± 14	264 ± 6	468 ± 16
		333 µg	17 ± 7	11 ± 5	36 ± 3	158 ± 12	269 ± 13	472 ± 16
		1000 µg	16 ± 2 P	8 ± 4 P	37 ± 2 P	102 ± 13 P	218 ± 4 P	444 ± 5 P
		2500 µg	11 ± 2 P	8 ± 2 P	29 ± 2 P	82 ± 9 P R	193 ± 16 P	365 ± 20 P
		5000 µg	10 ± 1 P	6 ± 2 P M	24 ± 1 P	45 ± 8 P M R	117 ± 12 P	282 ± 14 P
	2-AA	2.5 µg	515 ± 19	425 ± 51	3159 ± 128	3440 ± 100		
	2-AA	10.0 µg					2325 ± 43	2166 ± 109

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

P	Precipitate
R	Reduced background growth
M	Manual count

TABLE 2 Summary of Results Experiment II

Study Name: 1402001
Experiment: 1402001 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 13/04/2011
Date Counted: 19/04/2011

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	DMSO		15 ± 4	12 ± 4	26 ± 3	137 ± 8	240 ± 6	405 ± 8
	Untreated		17 ± 4	13 ± 4	30 ± 8	160 ± 3	293 ± 16	411 ± 20
	Azoxystrobin/	1 µg	14 ± 3	13 ± 1	27 ± 4	134 ± 6	247 ± 18	458 ± 55
	SYN545192 EC)	3 µg	16 ± 3	13 ± 2	21 ± 2	150 ± 30	275 ± 25	407 ± 21
	(A17961A)	10 µg	16 ± 1	14 ± 2	19 ± 4	133 ± 3	263 ± 27	394 ± 41
		33 µg	16 ± 5	13 ± 2	20 ± 3	127 ± 7	245 ± 14	376 ± 34
		100 µg	14 ± 2	8 ± 2	24 ± 2	110 ± 16	239 ± 28	371 ± 19
		333 µg	14 ± 1	7 ± 2 MR	13 ± 1	48 ± 8 MR	231 ± 33	386 ± 17
		1000 µg	2 ± 1 MRP	3 ± 1 PMR	13 ± 1 P	35 ± 5 PMR	148 ± 9 P	313 ± 17 P
		2500 µg	1 ± 1 MRP	1 ± 1 PMR	4 ± 2 PMR	17 ± 5 PMR	68 ± 6 P	209 ± 12 P
		5000 µg	0 ± 1 MRP	0 ± 1 PMR	2 ± 1 PMR	0 ± 0 PMR	7 ± 2 PMR	44 ± 8 PMR
	NaN3	10 µg	1787 ± 69			2200 ± 45		
	4-NOPD	10 µg			393 ± 9			
	4-NOPD	50 µg		65 ± 1				
	MMS	3.0 µL					2603 ± 33	2495 ± 84
With Activation	DMSO		21 ± 5	18 ± 1	35 ± 6	156 ± 14	249 ± 2	408 ± 24
	Untreated		27 ± 2	28 ± 6	44 ± 9	200 ± 14	294 ± 30	458 ± 21
	Azoxystrobin/	3 µg	20 ± 8	20 ± 4	34 ± 12	150 ± 4	266 ± 54	383 ± 53
	SYN545192 EC)	10 µg	18 ± 4	18 ± 7	43 ± 6	157 ± 29	239 ± 8	353 ± 26
	(A17961A)	33 µg	20 ± 3	19 ± 5	43 ± 3	164 ± 11	219 ± 18	370 ± 33
		100 µg	19 ± 3	19 ± 4	42 ± 1	138 ± 8	214 ± 8	331 ± 38
		333 µg	14 ± 3	16 ± 6	38 ± 3	127 ± 10	224 ± 36	377 ± 44
		1000 µg	14 ± 2 P	12 ± 3 PR	39 ± 2 P	85 ± 9 P	165 ± 4 P	302 ± 29 P
		2500 µg	9 ± 3 PMR	7 ± 1 PMR	25 ± 4 PR	36 ± 7 PMR	122 ± 6 P	238 ± 14 P
		5000 µg	3 ± 1 PMR	2 ± 1 PMR	12 ± 3 PMR	9 ± 2 PMR	51 ± 4 P	145 ± 22 P
	2-AA	2.5 µg	323 ± 31	288 ± 24	2026 ± 180	1982 ± 51		
	2-AA	10.0 µg					1568 ± 59	1974 ± 55

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth
P	Precipitate

TABLE 3 Pre-Experiment and Experiment I: 1402001VV Plate Incorporation

Study Name: 1402001
Experiment: 1402001 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 30/03/2011
Date Counted: 04/04/2011

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*14.3*	*5.0*	*1.1*	15, 9, 19
		10 µg	*14.7*	*1.5*	*1.1*	15, 16, 13
		33 µg	*14.0*	*2.0*	*1.1*	12, 16, 14
		100 µg	*11.3*	*1.2*	*0.9*	10, 12, 12
		333 µg	*14.0*	*1.7*	*1.1*	15, 15, 12
		1000 µg	*6.3*	*1.5*	*0.5*	8 P R M, 5 P R M, 6 P R M
		2500 µg	*4.3*	*0.6*	*0.3*	4 P M R, 5 P M R, 4 P M R
		5000 µg	*3.3*	*0.6*	*0.3*	4 P M R, 3 P M R, 3 P M R
	DMSO		*13.3*	*1.5*		13, 15, 12
	Untreated Control		*16.3*	*3.1*		19, 13, 17
TA 1537	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*9.3*	*2.5*	*1.0*	9, 12, 7
		10 µg	*10.7*	*2.3*	*1.1*	12, 8, 12
		33 µg	*13.3*	*0.6*	*1.4*	14, 13, 13
		100 µg	*9.3*	*2.5*	*1.0*	7, 12, 9
		333 µg	*6.7*	*2.1*	*0.7*	9, 5, 6
		1000 µg	*2.7*	*0.6*	*0.3*	3 P M R, 3 P M R, 2 P M R
		2500 µg	*2.0*	*1.0*	*0.2*	2 P M R, 1 P M R, 3 P M R
		5000 µg	*1.3*	*0.6*	*0.1*	1 P M R, 2 P M R, 1 P M R
	DMSO		*9.7*	*2.5*		10, 7, 12
	Untreated Control		*11.0*	*1.7*		12, 12, 9
TA 98	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*26.3*	*4.7*	*0.9*	30, 21, 28
		10 µg	*32.0*	*4.6*	*1.1*	36, 33, 27
		33 µg	*26.0*	*4.4*	*0.9*	23, 24, 31
		100 µg	*30.0*	*3.6*	*1.1*	29, 34, 27
		333 µg	*26.0*	*7.9*	*0.9*	23, 20, 35
		1000 µg	*16.3*	*3.1*	*0.6*	19 P, 17 P, 13 P
		2500 µg	*12.7*	*2.3*	*0.5*	10 P R, 14 P R, 14 P R
		5000 µg	*7.7*	*2.1*	*0.3*	7 P M R, 6 P M R, 10 P M R
	DMSO		*28.0*	*6.6*		29, 21, 34
	Untreated Control		*33.7*	*4.5*		29, 34, 38

Key to Plate Postfix Codes

P	Precipitate
R	Reduced background growth
M	Manual count

TABLE 3 Pre-Experiment and Experiment I: 1402001VV Plate Incorporation (Continued)

Study Name: 1402001
Experiment: 1402001 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 30/03/2011
Date Counted: 04/04/2011

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*158.7*	*6.8*	*0.9*	161, 164, 151
		10 µg	*180.3*	*13.6*	*1.0*	191, 185, 165
		33 µg	*161.3*	*26.5*	*0.9*	187, 163, 134
		100 µg	*147.7*	*11.9*	*0.9*	161, 138, 144
		333 µg	*76.7*	*13.2*	*0.4*	91, 65, 74
		1000 µg	*67.3*	*5.9*	*0.4*	63 P R, 65 P R, 74 P R
		2500 µg	*33.0*	*4.4*	*0.2*	31 P M R, 38 P M R, 30 P M R
		5000 µg	*12.0*	*3.0*	*0.1*	15 P M R, 9 P M R, 12 P M R
	DMSO		*172.0*	*8.5*		163, 173, 180
	Untreated Control		*181.0*	*7.2*		179, 175, 189
WP2 pKM101	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*246.7*	*22.8*	*1.1*	251, 267, 222
		10 µg	*241.3*	*14.2*	*1.0*	244, 254, 226
		33 µg	*253.3*	*8.4*	*1.1*	248, 263, 249
		100 µg	*245.0*	*21.9*	*1.1*	236, 229, 270
		333 µg	*210.7*	*18.6*	*0.9*	213, 228, 191
		1000 µg	*195.3*	*5.1*	*0.8*	191 P, 194 P, 201 P
		2500 µg	*161.3*	*7.0*	*0.7*	168 P, 162 P, 154 P
		5000 µg	*90.7*	*17.8*	*0.4*	111 P, 83 P, 78 P
	DMSO		*230.0*	*2.6*		229, 228, 233
	Untreated Control		*294.3*	*11.6*		282, 296, 305
WP2 uvrA pKM101	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*426.0*	*18.2*	*0.9*	405, 435, 438
		10 µg	*424.0*	*16.5*	*0.9*	405, 434, 433
		33 µg	*443.7*	*16.2*	*1.0*	454, 425, 452
		100 µg	*416.0*	*26.3*	*0.9*	397, 446, 405
		333 µg	*415.7*	*7.1*	*0.9*	417, 422, 408
		1000 µg	*375.7*	*9.2*	*0.8*	381 P, 365 P, 381 P
		2500 µg	*306.3*	*31.0*	*0.7*	319 P, 329 P, 271 P
		5000 µg	*147.0*	*16.5*	*0.3*	148 P, 163 P, 130 P
	DMSO		*460.3*	*53.0*		513, 461, 407
	Untreated Control		*438.3*	*9.3*		428, 441, 446

Key to Plate Postfix Codes

P	Precipitate
R	Reduced background growth
M	Manual count

TABLE 3 Pre-Experiment and Experiment I: 1402001VV Plate Incorporation (Continued)

Study Name: 1402001
Experiment: 1402001 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 30/03/2011
Date Counted: 04/04/2011

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**NaN3**	10 µg	*2237.7*	*14.2*	*167.8*	2254, 2230, 2229
TA 1537	**4-NOPD**	50 µg	*68.7*	*9.1*	*7.1*	70, 59, 77
TA 98	**4-NOPD**	10 µg	*370.3*	*1.5*	*13.2*	372, 370, 369
TA 100	**NaN3**	10 µg	*2264.0*	*34.8*	*13.2*	2232, 2259, 2301
WP2 pKM101	**MMS**	3.0 µL	*4109.3*	*14.0*	*17.9*	4123, 4110, 4095
WP2 uvrA pKM101	**MMS**	3.0 µL	*3862.3*	*92.8*	*8.4*	3967, 3830, 3790

Key to Positive Controls

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

TABLE 4 Pre-Experiment and Experiment I: 1402001VV Plate Incorporation

Study Name: 1402001
Experiment: 1402001 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 30/03/2011
Date Counted: 04/04/2011

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Azoxystrobin/**	3 µg	*20.0*	*3.6*	*1.1*	23, 16, 21
	SYN545192 EC	10 µg	*20.0*	*8.0*	*1.1*	20, 12, 28
	(A17961A)	33 µg	*16.7*	*3.5*	*0.9*	13, 20, 17
		100 µg	*16.7*	*4.7*	*0.9*	13, 22, 15
		333 µg	*16.7*	*7.0*	*0.9*	10, 16, 24
		1000 µg	*15.7*	*1.5*	*0.8*	14 P, 17 P, 16 P
		2500 µg	*11.3*	*2.1*	*0.6*	12 P, 13 P, 9 P
		5000 µg	*9.7*	*0.6*	*0.5*	10 P, 10 P, 9 P
	DMSO		*18.7*	*1.5*		17, 19, 20
	Untreated Control		*21.3*	*4.5*		21, 17, 26
TA 1537	**Azoxystrobin/**	3 µg	*11.7*	*3.8*	*1.0*	16, 9, 10
	SYN545192 EC	10 µg	*10.3*	*4.0*	*0.9*	15, 8, 8
	(A17961A)	33 µg	*11.7*	*5.1*	*1.0*	13, 16, 6
		100 µg	*9.7*	*5.5*	*0.9*	7, 6, 16
		333 µg	*11.0*	*4.6*	*1.0*	15, 12, 6
		1000 µg	*8.3*	*4.2*	*0.7*	7 P, 5 P, 13 P
		2500 µg	*8.3*	*1.5*	*0.7*	10 P, 8 P, 7 P
		5000 µg	*6.3*	*1.5*	*0.6*	5 P M, 8 P M, 6 P M
	DMSO		*11.3*	*2.9*		8, 13, 13
	Untreated Control		*13.7*	*3.5*		10, 17, 14
TA 98	**Azoxystrobin/**	3 µg	*33.3*	*0.6*	*1.1*	34, 33, 33
	SYN545192 EC	10 µg	*37.0*	*3.0*	*1.2*	37, 40, 34
	(A17961A)	33 µg	*40.0*	*2.0*	*1.3*	38, 40, 42
		100 µg	*37.3*	*3.5*	*1.2*	41, 37, 34
		333 µg	*36.0*	*2.6*	*1.1*	33, 38, 37
		1000 µg	*37.3*	*2.3*	*1.2*	36 P, 40 P, 36 P
		2500 µg	*28.7*	*1.5*	*0.9*	29 P, 30 P, 27 P
		5000 µg	*23.7*	*0.6*	*0.7*	23 P, 24 P, 24 P
	DMSO		*31.7*	*4.2*		33, 27, 35
	Untreated Control		*47.0*	*2.0*		49, 47, 45

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 4 Pre-Experiment and Experiment I: 1402001VV Plate Incorporation (Continued)

Study Name: 1402001
Experiment: 1402001 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 30/03/2011
Date Counted: 04/04/2011

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*168.0*	*7.0*	*1.0*	176, 165, 163
		10 µg	*170.3*	*21.5*	*1.0*	194, 152, 165
		33 µg	*169.7*	*8.5*	*1.0*	170, 178, 161
		100 µg	*165.3*	*13.9*	*1.0*	169, 150, 177
		333 µg	*158.3*	*12.3*	*0.9*	172, 148, 155
		1000 µg	*102.3*	*13.2*	*0.6*	88 P, 105 P, 114 P
		2500 µg	*82.0*	*8.7*	*0.5*	92 P R, 77 P R, 77 P R
		5000 µg	*45.3*	*7.5*	*0.3*	45 P M R, 53 P M R, 38 P M R
	DMSO		*167.7*	*15.6*		170, 151, 182
	Untreated Control		*182.7*	*21.8*		165, 176, 207
WP2 pKM101	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*281.7*	*18.6*	*1.0*	301, 280, 264
		10 µg	*284.7*	*12.9*	*1.0*	290, 270, 294
		33 µg	*276.3*	*8.6*	*1.0*	278, 284, 267
		100 µg	*264.3*	*5.5*	*1.0*	268, 267, 258
		333 µg	*269.3*	*13.3*	*1.0*	277, 277, 254
		1000 µg	*218.0*	*4.4*	*0.8*	221 P, 213 P, 220 P
		2500 µg	*192.7*	*15.9*	*0.7*	175 P, 197 P, 206 P
		5000 µg	*117.3*	*12.2*	*0.4*	104 P, 120 P, 128 P
	DMSO		*278.0*	*25.2*		307, 262, 265
	Untreated Control		*308.7*	*21.5*		327, 314, 285
WP2 uvrA pKM101	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*488.3*	*21.5*	*1.0*	483, 470, 512
		10 µg	*470.0*	*32.1*	*0.9*	505, 463, 442
		33 µg	*486.7*	*13.1*	*1.0*	472, 497, 491
		100 µg	*467.7*	*16.3*	*0.9*	479, 449, 475
		333 µg	*472.0*	*16.5*	*0.9*	463, 491, 462
		1000 µg	*444.0*	*5.3*	*0.9*	438 P, 446 P, 448 P
		2500 µg	*365.3*	*20.0*	*0.7*	386 P, 364 P, 346 P
		5000 µg	*282.3*	*14.5*	*0.6*	273 P, 299 P, 275 P
	DMSO		*504.7*	*16.9*		524, 497, 493
	Untreated Control		*494.7*	*19.6*		513, 474, 497

Key to Plate Postfix Codes

P	Precipitate
R	Reduced background growth
M	Manual count

TABLE 4 Pre-Experiment and Experiment I: 1402001VV Plate Incorporation (Continued)

Study Name: 1402001
Experiment: 1402001 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 30/03/2011
Date Counted: 04/04/2011

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**2-AA**	2.5 µg	*514.7*	*19.1*	*27.6*	536, 509, 499
TA 1537	**2-AA**	2.5 µg	*424.7*	*50.9*	*37.5*	411, 382, 481
TA 98	**2-AA**	2.5 µg	*3158.7*	*127.5*	*99.7*	3285, 3161, 3030
TA 100	**2-AA**	2.5 µg	*3440.0*	*99.6*	*20.5*	3497, 3325, 3498
WP2 pKM101	**2-AA**	10.0 µg	*2325.0*	*42.5*	*8.4*	2352, 2276, 2347
WP2 uvrA pKM101	**2-AA**	10.0 µg	*2166.3*	*108.8*	*4.3*	2213, 2244, 2042

Key to Positive Controls

2-AA	2-aminoanthracene

TABLE 5 Experiment II: 1402001Pre-Incubation

Study Name: 1402001
Experiment: 1402001 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 13/04/2011
Date Counted: 19/04/2011

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Azoxystrobin/ SYN545192 EC (A17961A)**	1 µg	*14.0*	*2.6*	*0.9*	17, 13, 12
		3 µg	*16.3*	*3.1*	*1.1*	17, 13, 19
		10 µg	*16.3*	*1.2*	*1.1*	15, 17, 17
		33 µg	*16.0*	*4.6*	*1.1*	12, 21, 15
		100 µg	*13.7*	*2.1*	*0.9*	16, 12, 13
		333 µg	*13.7*	*0.6*	*0.9*	14, 14, 13
		1000 µg	*2.3*	*0.6*	*0.2*	3 M R P, 2 M R P, 2 M R P
		2500 µg	*1.0*	*1.0*	*0.1*	2 M R P, 0 M R P, 1 M R P
		5000 µg	*0.3*	*0.6*	*0.0*	0 M R P, 1 M R P, 0 M R P
	DMSO		*15.0*	*4.4*		20, 13, 12
	Untreated Control		*17.0*	*3.6*		16, 14, 21
TA 1537	**Azoxystrobin/ SYN545192 EC (A17961A)**	1 µg	*12.7*	*1.2*	*1.0*	12, 12, 14
		3 µg	*13.3*	*2.3*	*1.1*	16, 12, 12
		10 µg	*14.0*	*1.7*	*1.1*	13, 13, 16
		33 µg	*13.3*	*2.3*	*1.1*	12, 16, 12
		100 µg	*8.3*	*1.5*	*0.7*	8, 7, 10
		333 µg	*6.7*	*2.1*	*0.5*	5 M R, 6 M R, 9 M R
		1000 µg	*3.0*	*1.0*	*0.2*	3 P M R, 4 P M R, 2 P M R
		2500 µg	*1.0*	*1.0*	*0.1*	0 P M R, 2 P M R, 1 P M R
		5000 µg	*0.3*	*0.6*	*0.0*	0 P M R, 1 P M R, 0 P M R
	DMSO		*12.3*	*3.5*		9, 12, 16
	Untreated Control		*13.3*	*4.0*		9, 17, 14
TA 98	**Azoxystrobin/ SYN545192 EC (A17961A)**	1 µg	*26.7*	*4.0*	*1.0*	23, 31, 26
		3 µg	*21.0*	*2.0*	*0.8*	23, 19, 21
		10 µg	*18.7*	*3.8*	*0.7*	23, 17, 16
		33 µg	*20.0*	*3.0*	*0.8*	17, 20, 23
		100 µg	*24.3*	*2.3*	*0.9*	23, 27, 23
		333 µg	*13.0*	*1.0*	*0.5*	13, 14, 12
		1000 µg	*13.3*	*0.6*	*0.5*	13 P, 14 P, 13 P
		2500 µg	*4.3*	*1.5*	*0.2*	3 P M R, 4 P M R, 6 P M R
		5000 µg	*1.7*	*1.2*	*0.1*	1 P M R, 3 P M R, 1 P M R
	DMSO		*26.0*	*2.6*		23, 28, 27
	Untreated Control		*30.3*	*7.5*		30, 38, 23

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth
P	Precipitate

TABLE 5 Experiment II: 1402001Pre-Incubation (Continued)

Study Name: 1402001
Experiment: 1402001 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 13/04/2011
Date Counted: 19/04/2011

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Azoxystrobin/**	1 µg	*134.0*	*6.0*	*1.0*	140, 134, 128
	SYN545192 EC	3 µg	*150.0*	*30.4*	*1.1*	123, 144, 183
	(A17961A)	10 µg	*132.7*	*3.1*	*1.0*	132, 130, 136
		33 µg	*127.3*	*6.5*	*0.9*	121, 127, 134
		100 µg	*110.3*	*15.5*	*0.8*	115, 93, 123
		333 µg	*48.3*	*7.5*	*0.4*	56 M R, 41 M R, 48 M R
		1000 µg	*35.3*	*4.5*	*0.3*	40 P M R, 35 P M R, 31 P M R
		2500 µg	*17.3*	*4.5*	*0.1*	17 P M R, 22 P M R, 13 P M R
		5000 µg	*0.0*	*0.0*	*0.0*	0 P M R, 0 P M R, 0 P M R
	DMSO		*137.0*	*8.0*		145, 129, 137
	Untreated Control		*160.3*	*3.1*		161, 157, 163
WP2	**Azoxystrobin/**	1 µg	*247.0*	*17.8*	*1.0*	261, 253, 227
pKM101	**SYN545192 EC**	3 µg	*275.3*	*24.9*	*1.1*	285, 294, 247
	(A17961A)	10 µg	*263.3*	*26.8*	*1.1*	241, 256, 293
		33 µg	*245.3*	*14.4*	*1.0*	237, 262, 237
		100 µg	*239.0*	*28.0*	*1.0*	211, 267, 239
		333 µg	*230.7*	*32.6*	*1.0*	216, 208, 268
		1000 µg	*148.0*	*9.2*	*0.6*	138 P, 156 P, 150 P
		2500 µg	*67.7*	*5.8*	*0.3*	71 P, 71 P, 61 P
		5000 µg	*7.3*	*1.5*	*0.0*	9 P M R, 6 P M R, 7 P M R
	DMSO		*240.0*	*6.0*		240, 234, 246
	Untreated Control		*292.7*	*16.3*		280, 287, 311
WP2	**Azoxystrobin/**	1 µg	*458.0*	*54.8*	*1.1*	432, 521, 421
uvrA	**SYN545192 EC**	3 µg	*407.3*	*20.7*	*1.0*	426, 411, 385
pKM101	**(A17961A)**	10 µg	*393.7*	*40.7*	*1.0*	398, 432, 351
		33 µg	*376.3*	*33.8*	*0.9*	339, 405, 385
		100 µg	*371.3*	*18.9*	*0.9*	367, 392, 355
		333 µg	*386.3*	*16.8*	*1.0*	368, 390, 401
		1000 µg	*313.0*	*16.6*	*0.8*	294 P, 320 P, 325 P
		2500 µg	*209.3*	*11.6*	*0.5*	211 P, 197 P, 220 P
		5000 µg	*44.0*	*8.0*	*0.1*	44 P M R, 36 P M R, 52 P M R
	DMSO		*405.3*	*7.6*		407, 412, 397
	Untreated Control		*411.3*	*19.7*		399, 434, 401

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth
P	Precipitate

TABLE 5 Experiment II: 1402001Pre-Incubation (Continued)

Study Name: 1402001 Study Code: Harlan CCR 1402001
Experiment: 1402001 HV2 Pre Date Plated: 13/04/2011
Assay Conditions: Date Counted: 19/04/2011

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**NaN3**	10 µg	*1787.0*	*69.3*	*119.1*	1867, 1748, 1746
TA 1537	**4-NOPD**	50 µg	*65.3*	*0.6*	*5.3*	65, 65, 66
TA 98	**4-NOPD**	10 µg	*393.3*	*9.1*	*15.1*	383, 397, 400
TA 100	**NaN3**	10 µg	*2199.7*	*45.4*	*16.1*	2158, 2193, 2248
WP2 pKM101	**MMS**	3.0 µL	*2603.0*	*33.1*	*10.8*	2632, 2567, 2610
WP2 uvrA pKM101	**MMS**	3.0 µL	*2495.3*	*84.3*	*6.2*	2407, 2504, 2575

Key to Positive Controls

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

TABLE 6 Experiment II: 1402001Pre-Incubation

Study Name: 1402001
Experiment: 1402001 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 13/04/2011
Date Counted: 19/04/2011

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*19.7*	*8.1*	*0.9*	15, 29, 15
		10 µg	*18.0*	*4.4*	*0.8*	16, 15, 23
		33 µg	*20.3*	*3.1*	*1.0*	23, 17, 21
		100 µg	*19.3*	*3.1*	*0.9*	20, 16, 22
		333 µg	*14.3*	*2.5*	*0.7*	14, 12, 17
		1000 µg	*13.7*	*1.5*	*0.6*	12 P, 14 P, 15 P
		2500 µg	*8.7*	*3.1*	*0.4*	8 P M R, 6 P M R, 12 P M R
		5000 µg	*2.7*	*0.6*	*0.1*	2 P M R, 3 P M R, 3 P M R
	DMSO		*21.3*	*4.5*		26, 21, 17
	Untreated Control		*27.3*	*1.5*		26, 29, 27
TA 1537	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*20.0*	*4.4*	*1.1*	22, 23, 15
		10 µg	*18.0*	*7.2*	*1.0*	20, 24, 10
		33 µg	*19.0*	*5.3*	*1.1*	23, 21, 13
		100 µg	*19.0*	*4.4*	*1.1*	22, 14, 21
		333 µg	*16.3*	*6.0*	*0.9*	10, 22, 17
		1000 µg	*12.3*	*3.1*	*0.7*	13 P R, 15 P R, 9 P R
		2500 µg	*6.7*	*1.2*	*0.4*	6 P M R, 6 P M R, 8 P M R
		5000 µg	*2.3*	*1.2*	*0.1*	3 P M R, 1 P M R, 3 P M R
	DMSO		*17.7*	*1.2*		17, 19, 17
	Untreated Control		*27.7*	*6.1*		29, 21, 33
TA 98	**Azoxystrobin/ SYN545192 EC (A17961A)**	3 µg	*34.3*	*11.8*	*1.0*	48, 28, 27
		10 µg	*43.0*	*6.2*	*1.2*	48, 36, 45
		33 µg	*42.7*	*2.5*	*1.2*	43, 40, 45
		100 µg	*42.3*	*0.6*	*1.2*	42, 42, 43
		333 µg	*38.0*	*3.5*	*1.1*	40, 40, 34
		1000 µg	*39.3*	*2.1*	*1.1*	40 P, 37 P, 41 P
		2500 µg	*25.3*	*3.8*	*0.7*	21 P R, 27 P R, 28 P R
		5000 µg	*11.7*	*2.5*	*0.3*	12 P M R, 14 P M R, 9 P M R
	DMSO		*35.3*	*5.5*		41, 30, 35
	Untreated Control		*44.3*	*9.0*		49, 34, 50

Key to Plate Postfix Codes

P	Precipitate
M	Manual count
R	Reduced background growth

TABLE 6 Experiment II: 1402001Pre-Incubation (Continued)

Study Name: 1402001
Experiment: 1402001 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1402001
Date Plated: 13/04/2011
Date Counted: 19/04/2011

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Azoxystrobin/**	3 µg	*150.3*	*3.5*	*1.0*	147, 154, 150
	SYN545192 EC	10 µg	*157.0*	*29.1*	*1.0*	187, 129, 155
	(A17961A)	33 µg	*164.3*	*10.5*	*1.1*	175, 164, 154
		100 µg	*137.7*	*7.5*	*0.9*	138, 145, 130
		333 µg	*127.0*	*9.5*	*0.8*	116, 133, 132
		1000 µg	*84.7*	*9.0*	*0.5*	79 P, 95 P, 80 P
		2500 µg	*35.7*	*6.5*	*0.2*	36 P M R, 42 P M R, 29 P M R
		5000 µg	*8.7*	*1.5*	*0.1*	9 P M R, 10 P M R, 7 P M R
	DMSO		*156.3*	*14.4*		173, 148, 148
	Untreated Control		*200.0*	*14.4*		204, 212, 184
WP2 pKM101	**Azoxystrobin/**	3 µg	*266.0*	*54.1*	*1.1*	308, 285, 205
	SYN545192 EC	10 µg	*239.3*	*8.3*	*1.0*	230, 242, 246
	(A17961A)	33 µg	*218.7*	*18.2*	*0.9*	199, 235, 222
		100 µg	*214.0*	*7.8*	*0.9*	205, 219, 218
		333 µg	*223.7*	*36.2*	*0.9*	262, 190, 219
		1000 µg	*165.0*	*3.6*	*0.7*	161 P, 168 P, 166 P
		2500 µg	*122.0*	*6.1*	*0.5*	119 P, 118 P, 129 P
		5000 µg	*51.0*	*4.0*	*0.2*	51 P, 47 P, 55 P
	DMSO		*249.3*	*2.1*		251, 247, 250
	Untreated Control		*294.0*	*29.5*		327, 270, 285
WP2 1 uvrA pKM10	**Azoxystrobin/**	3 µg	*383.0*	*52.8*	*0.9*	442, 367, 340
	SYN545192 EC	10 µg	*353.0*	*25.6*	*0.9*	326, 356, 377
	(A17961A)	33 µg	*369.7*	*32.8*	*0.9*	332, 385, 392
		100 µg	*330.7*	*37.6*	*0.8*	306, 312, 374
		333 µg	*376.7*	*43.5*	*0.9*	419, 379, 332
		1000 µg	*302.3*	*29.2*	*0.7*	336 P, 284 P, 287 P
		2500 µg	*238.3*	*14.0*	*0.6*	254 P, 234 P, 227 P
		5000 µg	*145.0*	*21.9*	*0.4*	136 P, 170 P, 129 P
	DMSO		*407.7*	*23.9*		427, 381, 415
	Untreated Control		*458.0*	*20.9*		472, 468, 434

Key to Plate Postfix Codes

P	Precipitate
M	Manual count
R	Reduced background growth

TABLE 6 Experiment II: 1402001Pre-Incubation (Continued)

Study Name: 1402001 Study Code: Harlan CCR 1402001
Experiment: 1402001 HV2 Pre Date Plated: 13/04/2011
Assay Conditions: Date Counted: 19/04/2011

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**2-AA**	2.5 µg	*323.3*	*31.1*	*15.2*	356, 294, 320
TA 1537	**2-AA**	2.5 µg	*287.7*	*24.2*	*16.3*	260, 305, 298
TA 98	**2-AA**	2.5 µg	*2025.7*	*180.5*	*57.3*	2172, 2081, 1824
TA 100	**2-AA**	2.5 µg	*1982.0*	*51.1*	*12.7*	2012, 1923, 2011
WP2 pKM101	**2-AA**	10.0 µg	*1567.7*	*59.1*	*6.3*	1511, 1563, 1629
WP2 uvrA pKM101	**2-AA**	10.0 µg	*1974.0*	*55.1*	*4.8*	1998, 1911, 2013

Key to Positive Controls

2-AA	2-aminoanthracene

TABLE 7 Batch control of S9

Lot R 081010		
Treatment	µL S9 / plate	Number of revertants in TA 98
Negative Control	0	45
	100	36
10 µg/plate 2-Aminoanthracene	0	37
	100	3226
10 µg/plate Benzo(a)pyrene	0	25
	100	236
Lot R 060111		
Treatment	µL S9 / plate	Number of revertants in TA 98
Negative Control	0	23
	100	25
10 µg/plate 2-Aminoanthracene	0	24
	100	3720
10 µg/plate Benzo(a)pyrene	0	21
	100	283

APPENDICES SECTION

APPENDIX 1 Historical Control Data

These data represent the laboratory´s historical control data from January 2009 until December 2009 representing approx. 550 experiments (WP2 uvrA pKM101 and WP2 pKM101the historical data are based on approx. 100 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA1535	Solvent control	16	3.37	8	38	19	4.37	10	41
	Untreated control	15	3.41	7	36	18	4.69	8	55
	Positive control	1886	242.09	663	2690	304	154.47	134	2404
TA1537	Solvent control	12	2.84	6	27	15	3.59	7	33
	Untreated control	12	3.23	5	27	16	3.92	7	31
	Positive control	101	28.30	58	440	227	67.24	68	498
TA98	Solvent control	30	5.26	15	52	38	6.58	16	59
	Untreated control	31	5.67	14	59	39	6.91	16	84
	Positive control	407	98.13	216	897	1586	454.52	198	3309
TA100	Solvent control	132	23.21	94	218	144	25.42	94	241
	Untreated control	142	21.88	85	226	154	25.80	94	239
	Positive control	1954	426.94	563	2844	2032	569.62	594	3724
WP2uvrA pKM 101	Solvent control	430	69.32	332	569	456	62.40	382	553
	Untreated control	432	61.78	352	564	486	65.39	409	607
	Positive control	3150	1082.68	1379	4911	2031	309.13	1480	2947
WP2 pKM 101	Solvent control	188	22.50	154	226	197	25.76	147	266
	Untreated control	202	22.55	168	251	240	27.94	201	303
	Positive control	2905	832.09	1657	4560	2095	432.92	1259	2983

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 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Harlan Cytotest Cell Research GmbH
Harlan 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	**2** Toxicity studies
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**3** Mutagenicity studies
6 Prüfungen zur Bestimmung von Rückständen	**6** Residues
8 Analytische Prüfungen an biologischen Materialien	**8** Analytical studies on biological materials

15.08. und 27. – 29.10.2008
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 **30. März 2009**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, LÄNDLICHEN RAUM UND VERBRAUCHERSCHUTZ 2

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

English name and address of the GLP Monitoring Authority: Hessian Ministry for Environment, Energy, Agriculture and Consumer Protection; Department II 10; P.O. Box 31 09; 65189 Wiesbaden

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

A17961A
Azoxystrobin/SYN545192 EC (100/050)
SMU0GL002

Batch Identification	**SMU0GL002**
Product Code	**A17961A**
Other Product Code(s)	ICI5504/SYN545192 EC (100/050)

Chemical Analysis
(Active Ingredient Content)

– **Identity of Azoxystrobin (ICI5504) ***	**confirmed**
– **Identity of SYN545192 ***	**confirmed**
– **Content of Azoxystrobin (ICI5504) ***	**99.9 g/l corresponding to 9.48 % w/w**
– **Content of SYN545192 ***	**50.3 g/l corresponding to 4.77 % w/w**
Methodology used for Characterization	HPLC

The Active Ingredient content is within the FAO limits.

Physical Analysis

– **Appearance**	**yellow to brown liquid**
– **Density ***	**1054 kg/m^3**

Stability:

– **Storage Temperature**	**< 30°C**
– **Recertification Date**	**End of July 2013**

If stored under the conditions given above, this test substance can be considered stable until the recertification date is reached.

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

Study number of batch Characterization: 121486

Authorization:

18 August 2010

E. Ebi
Analytical Development & Product Chemistry