Propiconazole/Fenpropidin

Propiconazole/Fenpropidin EC (A9050B) - *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay

Final Report

TEST GUIDELINE(S): OECD 471 (2020)

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PERFORMING LABORATORY: ICCR-Roßdorf GmbH
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SPONSOR(S): Syngenta Ltd.
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study performed in the test facility of ICCR-Roßdorf 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), in its currently valid version

OECD Principles of Good Laboratory Practice, (as revised in 1997), ENV/MC/CHEM(98)17

EC Commission Directive 2004/10/EC

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), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

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

Dr. Steffi Chang
Study Director Bacterial Systems ..... Date: 19 July 2021

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QUALITY ASSURANCE STATEMENT

ICCR Study Number: 2166400
Test substance: Propiconazole/Fenpropidin EC (A9050B)
Study director: Dr. Steffi Chang
Study Title: Propiconazole/Fenpropidin EC (A9050B) - *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay

Study based activities at the Test Facility ICCR-Roßdorf GmbH were audited and inspected. The details of these audits and inspections are given below.

Type of Inspection	Date(s) of Inspection	Date Reporting to Study Director, Test Facility Management
Study Plan Verification	03 May 2021	03 May 2021
Process – based Test Item Preparation	05 May 2021	05 May 2021
Report Audit	23 June 2021	24 June 2021

General facilities and activities where this study was conducted were inspected on an annual basis and results are reported to the relevant responsible person and Management.

The statement is to confirm, that this report reflects the raw data.

S. Ebert

Sabine Ebert
Quality Assurance Auditor
ICCR-Roßdorf GmbH

19 July 2021
Date

PROJECT STAFF SIGNATURE

Study Director

Dr. Steffi Chang

.....

Date: 19 July 2021

GENERAL INFORMATION

Contributors
The following contributed to this report in the capacities indicated:

Name	Title
Dr. Steffi Chang	Study Director
Dr. Markus Schulz	Test Facility Management
Frauke Hermann	Head of Quality Assurance Unit
Merielen Pontes	Syngenta Study Manager

Study Dates
Study initiation date: 03 May 2021
Experimental start date: 07 May 2021
Experimental completion date: 31 May 2021

Deviations from the Guidelines
None

Retention of Samples
None

Performing Laboratory Test Substance Reference Number
S 2156211

Other
ICCR-Roßdorf GmbH will archive:
Records and documentation relating to this study will be maintained in the archives of ICCR-Roßdorf GmbH for a period of 4 years from the date on which the Study Director signs the final report. This will include electronic and paper raw data, and report that support the reconstruction of the study.

At termination of the aforementioned period, the records and documentation will be transferred to the GLP compliant Archive of Rhenus Archiv Services GmbH, Frankfurt am Main for further archiving up to a total archiving period of 15 years.

A sample of the test substance will not be archived.

ICCR Roßdorf GmbH will retain in its archive a copy of the study plan and final report, and any amendments indefinitely.

Deviations from the study plan
There were no deviations (unplanned changes) from the study plan.

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 propiconazole/fenpropidin EC (A9050B) to induce gene mutations in the plate incorporation test (Experiment I and Ia) and the pre-incubation test (Experiment II) using the *Salmonella typhimurium (S. typhimurium)* strains TA1535, TA1537, TA98, and TA100, and the *Escherichia coli (E. coli)* strains WP2 *uvrA* (pKM101) and WP2 (pKM101).

1.2 Results

In Experiment I reduced background growth was observed on the plates incubated with the test substance in strain TA1535 from 1000 to 5000 µg/plate in the presence and absence of S9 mix.

Cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in all strains with and without metabolic activation (S9 mix).

No relevant increase in revertant colony numbers of any of the six tester strains was observed following treatment with propiconazole/fenpropidin EC (A9050B) at any concentration, neither in the presence nor absence of metabolic activation (S9 mix). There was also no observed 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, which showed a distinct increase of induced revertant colonies consistent with the laboratory's historical control data demonstrated the sensitivity of the test system and the efficacy of the S9 mix. Each batch of S9 was also tested with 2 pro-mutagens, benzo(a)pyrene and 2-aminoanthracene.

1.3 Conclusion

In conclusion, it can be stated that during the described mutagenicity tests and under the experimental conditions reported, propiconazole/fenpropidin EC (A9050B) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, propiconazole/fenpropidin EC (A9050B) is considered to be non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

2.0 INTRODUCTION

2.1 Purpose

These experiments were performed to assess the potential of the test substance to induce gene mutations by means of the *S. typhimurium* and *E. coli* reverse mutation assay. Experiment I was performed as a plate incorporation assay. Since the acceptance criterium of four concentrations showing no signs of cytotoxicity was not met in Experiment I in strain WP2 (pKM101) without S9 mix, this part of Experiment I was repeated with adequately spaced concentrations in parallel to Experiment II. The repeated experiment is reported as Experiment Ia. Since a negative result was obtained in Experiment I, 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 *E. 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 and pre-incubation method the bacteria are exposed to the test substance with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a concentration response effect at least seven concentrations with adequately spaced intervals were tested. The maximum concentration was 5000 µg/plate in Experiment I and 2500 µg/plate in Experiment Ia and Experiment II.

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

2.2 Test Guideline(s)

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

"Ninth Addendum to OECD Guidelines for Testing of Chemicals", Section 4, No. 471: "Bacterial Reverse Mutation Test", corrected June 26, 2020

3.0 MATERIALS AND METHODS

3.1 Test Substance

Information as provided by the Sponsor.

Identification:	Propiconazole/Fenpropidin EC (A9050B)
Batch:	STH001-015-001
Content of propiconazole (sum of CGA93590 and CGA93591):	13.1% corresponding to 125 g/L
Content of fenpropidin:	28.8% corresponding to 274 g/L
Appearance:	Light yellow liquid
Recertification Date:	30 September 2025
Storage Conditions:	At room temperature
Stability in Solvent:	Not indicated by the Sponsor

The test substance concentrations were not adjusted for the content of propiconazole or fenpropidin.

On the day of the experiment (immediately before use), the test substance was dissolved in ethanol (purity ≥ 99.8%). The solvent was chosen as the most suitable solvent compared to water and DMSO according to its solubilisation properties and its relative non-toxicity to the bacteria (2).

All formulations were prepared freshly before treatment and used within two hours of preparation. The formulation was assumed to be stable for this period unless specified otherwise by the Sponsor.

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, 69042 Heidelberg, Germany
Batch No.:	150564
Purity:	≥ 99%
Dissolved in:	Deionised water
Concentration:	10 µg/plate

Strains:	TA1537, TA98
Name:	4-nitro-o-phenylene-diamine, (4-NOPD)
Supplier:	Sigma-Aldrich, 82024 Taufkirchen, Germany
Batch No.:	MKBM 5257V
Purity:	≥ 98%
Dissolved in:	DMSO (purity >99 %, Fisher Leics LE11 5RG, United Kingdom)
Concentration:	10 µg/plate in strain TA 98, 50 µg/plate in strain TA 1537

Strains:	WP2 *uvrA* (pKM101), WP2 (pKM101)
Name:	Methyl methane sulfonate, (MMS)
Supplier:	Sigma-Aldrich, 82024 Taufkirchen, Germany
Batch No.:	MKCL 6261
Purity:	≥ 99%
Dissolved in:	Deionised water
Concentration:	2.0 µL/plate

With metabolic activation

Strains:	TA1535, TA1537, TA98, TA100, WP2 *uvrA* (pKM101), WP2 (pKM 101)
Name:	2-aminoanthracene, (2-AA)
Supplier:	Sigma-Aldrich, 82024 Taufkirchen, Germany
Batch No.:	STBG 0630V
Purity:	≥ 96%
Dissolved in:	DMSO (purity > 99 %, Fisher Leics LE11 5RG, United Kingdom)
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 is unknown but a mutagenic response in the expected range is sufficient evidence of biological activity.

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 last 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 TA98 and TA100 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 summarized, the mutations of the *S. typhimurium* and *E. coli* strains used in this study can be described as follows:

Strains	**Genotype**	**Type of mutations indicated**
Salmonella typhimurium		
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 *S.typhimurium* and *E. coli* strains regarding the membrane permeability and ampicillin resistance; UV sensitivity, and amino acid requirement as well as normal spontaneous mutation rates is performed by ICCR-Roßdorf GmbH according to Ames *et al.* (5), Maron and Ames (3), and Mortelmans and Riccio (7). 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 WP2 (pKM101) 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 (Fisher Leics, LE11 5RG, United Kingdom) in liquid nitrogen.

3.3.3 Precultures

The thawed bacterial suspension was transferred into 250 mL Erlenmeyer flasks containing nutrient medium (50 mL). A solution of ampicillin (50 µL, 25 µg/mL) was added to the strains TA98, TA100, WP2 *uvrA* (pKM101), and WP2 (pKM101). This nutrient medium contains per liter:

8 g Nutrient Broth (MERCK, 64293 Darmstadt, Germany)
5 g NaCl (MERCK, 64293 Darmstadt, Germany)

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

Plates with selective agar (without Histidine/Tryptophan) were used.

3.3.5 Overlay agar

The overlay agar contained per litre:

for *Salmonella* strains:	for *Escherichia coli* strains:
7.0 g Agar Agar*	7.0 g Agar Agar*
6.0 g NaCl*	6.0 g NaCl*
10.5 mg L-Histidine×HCl×H_2O*	10.2 mg Tryptophan*
12.2 mg Biotin*	

* (MERCK, 64293 Darmstadt, Germany)

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 ICCR-Roßdorf GmbH)

Phenobarbital/ß-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Wistar rats (RjHan:WI; weight approx. 220 – 320 g, Janvier Labs, 53941 Saint-Berthevin Cedex, France) induced by peroral administration of 80 mg/kg b.w. phenobarbital (Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) and by peroral administrations of ß-naphthoflavone (Acros Organics, 2440 Geel, Belgium) each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were 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. Certificates of original preparation and recertification of the S9 fraction are given in Appendix 3.

The protein concentration in the S9 preparation was 31.2 mg/mL (lot no. 291020D) in Experiment I and Experiment II. To ensure that the characteristics of the S9 batch have not changed, the batch was recertified on 10 May 2021. Experiment I (performed 07 May 2021) was conducted prior to the recertification date, Experiment II (performed 26 May 2021) after the recertification date. Experiment Ia was performed without S9 mix only.

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 were 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 Cytotoxicity

To evaluate the cytotoxicity of the test substance a pre-experiment was performed with all strains. Eight concentrations were tested for cytotoxicity and mutation induction each with three replicate plates. The experimental conditions in this pre-experiment are described in section 3.7 (plate incorporation test).

Cytotoxicity of the test substance 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 is reported as the Main Experiment I since the criteria mentioned in Section 3.8.2 Acceptability of the Assay were met.

3.6 Concentration Selection

In the pre-experiment the concentration range of the test substance was 3 - 5000 µg/plate. The pre-experiment is reported as Experiment I. Based on strong cytotoxic effects, observed in Experiment I, a minimum of seven concentrations was tested in Experiment II. Since the acceptance criterium of four concentrations showing no signs of cytotoxic effects was not fulfilled in Experiment I in strain WP2 (pKM101) without S9 mix, this part of Experiment I was repeated with adequately spaced concentrations in parallel to Experiment II (reported as Experiment Ia). 2500 µg/plate was chosen as the maximal concentration in Experiment Ia and Experiment II. The concentration range included two logarithmic decades.

The following concentrations were tested in Experiment Ia:
1; 3; 10; 33; 100; 333; 1000 and 2500 µg/plate

The following concentrations were tested in Experiment II:
Strain WP2 (pKM101); 1; 3; 10; 33; 100; 333; 1000 and 2500 µg/plate
The remaing strains: 3; 10; 33; 100; 333; 1000 and 2500 µg/plate

3.7 Experimental Performance

For each strain and concentration 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 concentration, 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; OD = 1.0 - 1.2; wavelength = 500 nm; approx. $8x10^8$ cells/mL),
2000 µL Overlay agar

For the pre-incubation method test solution (50 µL) (solvent) or 100 µL reference mutagen solution (positive control), S9 mix / S9 mix substitution buffer* (500 µL) and bacteria suspension (100 µL) were mixed in a test tube and incubated at 37 C ± 1.5° C for 60 minutes. After pre-incubation overlay agar (2.0 mL, 45 °C) was added to each tube. The mixture was poured on selective agar plates.

After solidification the plates were incubated upside down for 72 hours at 37 C ± 1.5° C in the dark, plates were then stored at 4 °C until counted (6).

In parallel to each test a sterile control of the test substance was performed and documented in the raw data. Therefore, stock solution (100 µL in Experiment I and Ia; 50 µL in Experiment II) and S9 mix / S9 mix substitution buffer* (500 µL) were mixed with overlay agar (2.0 mL) and poured on minimal agar plates.

* 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 a Petri Viewer with the software program Ames Study Manager (see section 3.9, Major computerized systems). The evaluation unit was connected to a PC with printer to print out the individual values, 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). The print outs are kept with the raw data. Due to precipitation of the test item and reduced background growth some test groups were scored manually (as indicated on data tables).

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 the historical data
- the positive control substances should produce an increase above the threshold of twofold (strains TA 98, TA 100, WP2 uvrA (pKM101, and WP2 (pKM101))) or threefold (strains TA 1535 and TA 1537) the revertant colony count of the corresponding solvent control;
- a minimum of five analysable concentrations should be present with at least four 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 substance is considered as a mutagen if a biologically relevant increase in the number of revertants of twofold or above (strains TA 98, TA 100, WP2 uvrA (pKM101), and WP2 (pKM101)) or of threefold or above (strains TA 1535 and TA 1537) the spontaneous mutation rate of the corresponding solvent control is observed (1).

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

An increase of revertant colonies equal or above the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A concentration 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.

3.9 Major Computerized System

Petri Viewer Sorcerer Colony Counter 3.0 (Instem, Suffolk IP33 3TA, UK) with the software program Ames Study Manager (v1.24) and Ames Archive Manager (v1.01).

4.0 RESULTS AND DISCUSSION

The test substance, propiconazole/fenpropidin EC (A9050B), was assessed for its potential to induce gene mutations in the plate incorporation test (Experiment I and Ia) and the pre-incubation test (Experiment II) using *S. typhimurium* strains TA1535, TA1537, TA98, and TA100, and the *E. coli* strains WP2 (pKM101) and WP2 *uvrA* (pKM101).

In the pre-experiment the concentration range of the test substance was 3 - 5000 µg/plate. The pre-experiment is reported as Experiment I. Based on strong cytotoxic effects, observed in Experiment I, a minimum of seven concentrations was tested in Experiment II. Since the acceptance criterium of four concentrations showing no signs of cytotoxic effects was not fulfilled in Experiment I in strain WP2 (pKM101) without S9 mix, this part of Experiment I was repeated with adequately spaced concentrations in parallel to Experiment II (reported as Experiment Ia). 2500 µg/plate was chosen as the maximal concentration in Experiment Ia and Experiment II.

The assay was performed with and without liver microsomal activation in Experiment I and II. Experiment Ia was performed without S9 mix only. Each concentration, including the controls, was tested in triplicate. The concentration range included two logarithmic decades. The test substance was tested at the following concentrations:

Pre-Experiment/Experiment I:	3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment Ia:	1; 3; 10; 33; 100; 333; 1000 and 2500 µg/plate
Experiment II:	
Strain WP2 (pKM101);	1; 3; 10; 33; 100; 333; 1000 and 2500 µg/plate
The remaing strains:	3; 10; 33; 100; 333; 1000 and 2500 µg/plate

The test substance precipitated in the overlay agar in the test tubes from 1000 µg/plate up to the highest investigated concentration. Precipitation of the test item in the overlay agar on the incubated agar plates was observed in Experiment I from 1000 to 5000 µg/plate in the absence of S9 mix and from 2500 to 5000 µg/plate in the presence of S9 mix, in Experiment Ia from 1000 to 2500 µg/plate in the absence of S9 mix and in Experiment II at 2500 µg/plate in the absence of S9 mix and from 1000 to 2500 µg/plate in the presence of S9 mix. The undissolved particles had no influence on the data recording.

In Experiment I reduced background growth was observed on the plates incubated with the test substance in strain TA1535 from 1000 to 5000 µg/plate in the presence and absence of S9 mix.

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

Strain	Experiment I		Experiment Ia	Experiment II	
	without S9 mix	with S9 mix	without S9 mix	without S9 mix	with S9 mix
TA1535	2500 – 5000	2500 – 5000	n.p.	1000 - 2500	1000 - 2500
TA1537	2500 – 5000	2500 – 5000	n.p.	1000 – 2500	1000 – 2500
TA98	1000 – 5000	2500 – 5000	n.p.	333 – 2500	1000 – 2500
TA100	1000 – 5000	1000 – 5000	n.p.	333 – 2500	333 – 2500
WP2 (pKM101)	100 – 5000	1000 – 5000	100 – 2500	100 – 2500	333 – 2500
WP2 *uvrA* (pKM101)	333 – 5000	1000 – 5000	n.p.	1000 - 2500	1000 - 2500

/ = no cytotoxic effects, evident as a reduction in the number of revertants (below the induction factor of 0.5)
n.p. = not performed

No substantial increase in revertant colony numbers in any of the six tester strains was observed following treatment with propiconazole/fenpropidin EC (A9050B) at any concentration, 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 in induced revertant colonies demonstrating the correct performance of the assay and the activity of the S9, which was also independently validated.

5.0 CONCLUSIONS

In conclusion, it can be stated that during the described mutagenicity tests and under the experimental conditions reported, propiconazole/fenpropidin EC (A9050B) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, propiconazole/fenpropidin EC (A9050B) is considered to be non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

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   The bacterial tryptophan reverse mutation assay with Escherichia coli WP2
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TABLES SECTION

TABLE 1 Summary of Results Pre-Experiment/Experiment I

Study Name: 2166400
Experiment: 2166400 VV Plate
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 07.05.2021
Date Counted: 11.05.2021

Metabolic Activation	Test Group	Concentration (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	Ethanol		12 ± 4	12 ± 2	34 ± 6	91 ± 6	214 ± 30	384 ± 4
	Untreated		13 ± 2	17 ± 4	38 ± 11	107 ± 3	249 ± 28	393 ± 30
	Propiconazole/	3 µg	11 ± 1	11 ± 4	37 ± 5	103 ± 11	196 ± 32	422 ± 35
	Fenpropidin EC	10 µg	12 ± 4	11 ± 1	31 ± 8	103 ± 12	191 ± 9	387 ± 33
	(A9050B)	33 µg	14 ± 2	10 ± 1	31 ± 3	99 ± 3	168 ± 20	391 ± 12
		100 µg	12 ± 2	12 ± 2	35 ± 7	88 ± 7	95 ± 33	365 ± 19
		333 µg	12 ± 4	9 ± 3	22 ± 1	88 ± 7	47 ± 6	131 ± 6
		1000 µg	16 ± 3 P M R	7 ± 2 P	3 ± 1 P	1 ± 1 P	35 ± 4 P	99 ± 8 P
		2500 µg	2 ± 1 P R	2 ± 1 P	0 ± 1 P	0 ± 0 P	0 ± 1 P	6 ± 1 P
		5000 µg	0 ± 0 P R	1 ± 1 P	0 ± 0 P	0 ± 1 P	0 ± 0 P	0 ± 1 P
	NaN3	10 µg	1116 ± 41			1890 ± 82		
	4-NOPD	10 µg			397 ± 5			
	4-NOPD	50 µg		78 ± 5				
	MMS	2.0 µL					2587 ± 24	2307 ± 47
With Activation	Ethanol		14 ± 4	11 ± 1	43 ± 12	96 ± 3	220 ± 18	430 ± 7
	Untreated		17 ± 4	12 ± 3	43 ± 9	114 ± 13	241 ± 17	413 ± 39
	Propiconazole/	3 µg	16 ± 1	12 ± 4	50 ± 6	114 ± 10	222 ± 48	439 ± 18
	Fenpropidin EC	10 µg	14 ± 2	13 ± 4	53 ± 11	110 ± 6	179 ± 9	427 ± 20
	(A9050B)	33 µg	17 ± 2	13 ± 2	50 ± 5	125 ± 5	196 ± 9	429 ± 26
		100 µg	15 ± 3	15 ± 2	41 ± 4	115 ± 10	163 ± 14	403 ± 17
		333 µg	19 ± 1	14 ± 3	49 ± 9	86 ± 3	132 ± 7	314 ± 46
		1000 µg	14 ± 2 R	8 ± 2	33 ± 8	10 ± 3	94 ± 20	113 ± 8
		2500 µg	3 ± 1 P R	1 ± 1 P	3 ± 1 P	0 ± 1 P	13 ± 3 P	102 ± 7 P
		5000 µg	0 ± 0 P R	0 ± 1 P	0 ± 0 P	0 ± 1 P	1 ± 1 P	1 ± 1 P
	2-AA	2.5 µg	251 ± 21	398 ± 20	2372 ± 175	3603 ± 200		
	2-AA	10.0 µg					916 ± 37	1436 ± 103

Key to Positive Controls		Key to Plate Postfix Codes	
NaN3	sodium azide	P	Precipitate
2-AA	2-aminoanthracene	M	Manual count
4-NOPD	4-nitro-o-phenylene-diamine	R	Reduced background growth
MMS	methyl methane sulfonate		

TABLE 2 Summary of Results Experiment Ia

Study Name: 2166400
Experiment: 2166400 HV1a Plate
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 26.05.2021
Date Counted: 31.05.2021

Metabolic Activation	Test Group	Concentration (per plate)	Revertant Colony Counts (Mean ±SD)
			WP2 pKM101
Without Activation	Ethanol		252 ± 18
	Untreated		290 ± 33
	Propiconazole/ Fenpropidin EC (A9050B)	1 µg	267 ± 7
		3 µg	253 ± 26
		10 µg	254 ± 15
		33 µg	264 ± 19
		100 µg	76 ± 11
		333 µg	30 ± 10
		1000 µg	0 ± 0 [P]
		2500 µg	0 ± 0 [P]
	MMS	2.0 µL	3045 ± 185

Key to Positive Controls

MMS methyl methane sulfonate

Key to Plate Postfix Codes

P Precipitate

TABLE 3 Summary of Results Experiment II

Study Name: 2166400
Experiment: 2166400 HV2 Pre
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 26.05.2021
Date Counted: 31.05.2021

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	Ethanol		15 ± 5	11 ± 3	37 ± 1	98 ± 7	262 ± 12	339 ± 21
	Untreated		14 ± 2	11 ± 3	31 ± 4	108 ± 11	300 ± 8	368 ± 28
	Propiconazole/	1 µg					283 ± 14	
	Fenpropidin EC	3 µg	13 ± 6	12 ± 3	34 ± 8	116 ± 11	247 ± 11	340 ± 24
	(A9050B)	10 µg	13 ± 3	12 ± 2	25 ± 7	108 ± 24	270 ± 17	327 ± 16
		33 µg	15 ± 5	13 ± 2	35 ± 10	96 ± 3	274 ± 13	354 ± 2
		100 µg	8 ± 3	14 ± 3	29 ± 7	69 ± 8	89 ± 6	269 ± 21
		333 µg	9 ± 2	7 ± 3	5 ± 1	28 ± 7	0 ± 1	215 ± 12
		1000 µg	0 ± 0	1 ± 1	0 ± 1	0 ± 0	0 ± 0	3 ± 1
		2500 µg	0 ± 0 P	0 ± 0 P	0 ± 0 P	0 ± 0 P	0 ± 0 P	0 ± 0 P
	NaN3	10 µg	1161 ± 90			1771 ± 51		
	4-NOPD	10 µg			363 ± 22			
	4-NOPD	50 µg		68 ± 10				
	MMS	2.0 µL					2735 ± 64	2332 ± 81
With Activation	Ethanol		17 ± 3	15 ± 2	51 ± 7	117 ± 6	262 ± 20	404 ± 25
	Untreated		12 ± 3	14 ± 4	35 ± 5	109 ± 5	337 ± 14	425 ± 25
	Propiconazole/	1 µg					276 ± 13	
	Fenpropidin EC	3 µg	17 ± 5	12 ± 2	53 ± 9	112 ± 9	274 ± 9	404 ± 25
	(A9050B)	10 µg	14 ± 4	13 ± 6	48 ± 6	120 ± 15	274 ± 17	396 ± 17
		33 µg	15 ± 4	13 ± 5	45 ± 8	120 ± 13	240 ± 12	408 ± 19
		100 µg	10 ± 3	14 ± 4	45 ± 10	90 ± 11	291 ± 9	436 ± 20
		333 µg	10 ± 4	12 ± 2	39 ± 3	15 ± 3	82 ± 6	196 ± 21
		1000 µg	6 ± 2 P	2 ± 1 P	1 ± 1 P	0 ± 0 P	49 ± 8 P	144 ± 8 P
		2500 µg	0 ± 0 P	0 ± 0 P	0 ± 0 P	0 ± 0 P	2 ± 0 P	31 ± 12 P
	2-AA	2.5 µg	250 ± 19	335 ± 8	2654 ± 459	3825 ± 381		
	2-AA	10.0 µg					973 ± 47	1415 ± 56

Key to Positive Controls		Key to Plate Postfix Codes	
NaN3	sodium azide	P	Precipitate
2-AA	2-aminoanthracene		
4-NOPD	4-nitro-o-phenylene-diamine		
MMS	methyl methane sulfonate		

TABLE 4 Pre-Experiment/Experiment I: 2166400 VV Plate Incorporation Without Metabolic Activation

Study Name: 2166400
Experiment: 2166400 VV Plate
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 07.05.2021
Date Counted: 11.05.2021

Without metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Propiconazole/**	3 µg	*11.0*	*1.0*	*0.9*	12, 10, 11
	Fenpropidin EC	10 µg	*11.7*	*4.0*	*1.0*	7, 14, 14
	(A9050B)	33 µg	*14.3*	*2.1*	*1.2*	15, 16, 12
		100 µg	*12.0*	*1.7*	*1.0*	14, 11, 11
		333 µg	*11.7*	*3.8*	*1.0*	9, 16, 10
		1000 µg	*16.3*	*2.5*	*1.4*	14 P M R, 16 P M R, 19 P M R
		2500 µg	*1.7*	*0.6*	*0.1*	2 P R, 2 P R, 1 P R
		5000 µg	*0.0*	*0.0*	*0.0*	0 P R, 0 P R, 0 P R
	Ethanol		*12.0*	*3.6*		11, 16, 9
	Untreated		*12.7*	*2.1*		11, 15, 12
TA 1537	**Propiconazole/**	3 µg	*11.0*	*4.0*	*0.9*	15, 11, 7
	Fenpropidin EC	10 µg	*11.0*	*1.0*	*0.9*	12, 11, 10
	(A9050B)	33 µg	*10.3*	*1.2*	*0.8*	11, 11, 9
		100 µg	*12.3*	*1.5*	*1.0*	12, 14, 11
		333 µg	*8.7*	*2.5*	*0.7*	11, 6, 9
		1000 µg	*6.7*	*2.1*	*0.5*	9 P, 5 P, 6 P
		2500 µg	*1.7*	*0.6*	*0.1*	2 P, 2 P, 1 P
		5000 µg	*0.7*	*0.6*	*0.1*	1 P, 0 P, 1 P
	Ethanol		*12.3*	*1.5*		11, 12, 14
	Untreated		*17.3*	*3.5*		21, 17, 14
TA 98	**Propiconazole/**	3 µg	*37.3*	*5.1*	*1.1*	43, 33, 36
	Fenpropidin EC	10 µg	*31.0*	*7.9*	*0.9*	25, 28, 40
	(A9050B)	33 µg	*31.0*	*2.6*	*0.9*	33, 28, 32
		100 µg	*35.0*	*7.0*	*1.0*	38, 27, 40
		333 µg	*22.0*	*1.0*	*0.6*	22, 21, 23
		1000 µg	*3.3*	*1.2*	*0.1*	4 P, 4 P, 2 P
		2500 µg	*0.3*	*0.6*	*0.0*	0 P, 0 P, 1 P
		5000 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*34.3*	*5.5*		28, 38, 37
	Untreated		*37.7*	*10.6*		36, 28, 49

Key to Plate Postfix Codes

P	Precipitate
M	Manual count
R	Reduced background growth

Study Name: 2166400
Experiment: 2166400 VV Plate
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 07.05.2021
Date Counted: 11.05.2021

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Propiconazole/**	3 µg	*103.0*	*11.3*	*1.1*	110, 109, 90
	Fenpropidin EC	10 µg	*103.3*	*11.7*	*1.1*	93, 116, 101
	(A9050B)	33 µg	*99.3*	*2.9*	*1.1*	96, 101, 101
		100 µg	*88.3*	*6.8*	*1.0*	96, 86, 83
		333 µg	*88.3*	*6.5*	*1.0*	95, 88, 82
		1000 µg	*0.7*	*0.6*	*0.0*	1 P, 0 P, 1 P
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
		5000 µg	*0.3*	*0.6*	*0.0*	1 P, 0 P, 0 P
	Ethanol		*91.3*	*6.4*		94, 96, 84
	Untreated		*107.0*	*3.0*		110, 107, 104
WP2	**Propiconazole/**	3 µg	*195.7*	*31.5*	*0.9*	227, 196, 164
pKM101	**Fenpropidin EC**	10 µg	*190.7*	*9.0*	*0.9*	201, 186, 185
	(A9050B)	33 µg	*167.7*	*19.5*	*0.8*	190, 159, 154
		100 µg	*95.0*	*33.5*	*0.4*	70, 82, 133
		333 µg	*47.3*	*6.4*	*0.2*	51, 51, 40
		1000 µg	*34.7*	*3.5*	*0.2*	31 P, 35 P, 38 P
		2500 µg	*0.3*	*0.6*	*0.0*	0 P, 0 P, 1 P
		5000 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*214.3*	*30.1*		180, 236, 227
	Untreated		*249.0*	*27.9*		280, 241, 226
WP2	**Propiconazole/**	3 µg	*422.3*	*35.4*	*1.1*	462, 394, 411
uvrA	**Fenpropidin EC**	10 µg	*387.3*	*32.7*	*1.0*	357, 422, 383
pKM101	**(A9050B)**	33 µg	*391.0*	*12.1*	*1.0*	384, 384, 405
		100 µg	*364.7*	*18.8*	*0.9*	343, 377, 374
		333 µg	*131.3*	*5.7*	*0.3*	125, 136, 133
		1000 µg	*99.3*	*8.0*	*0.3*	100 P, 91 P, 107 P
		2500 µg	*5.7*	*0.6*	*0.0*	5 P, 6 P, 6 P
		5000 µg	*0.3*	*0.6*	*0.0*	0 P, 1 P, 0 P
	Ethanol		*384.0*	*4.0*		388, 384, 380
	Untreated		*392.7*	*29.5*		405, 414, 359
TA 1535	**NaN3**	10 µg	*1116.3*	*41.1*	*93.0*	1151, 1071, 1127
TA 1537	**4-NOPD**	50 µg	*77.7*	*5.1*	*6.3*	79, 72, 82
TA 98	**4-NOPD**	10 µg	*396.7*	*5.1*	*11.6*	401, 398, 391
TA 100	**NaN3**	10 µg	*1890.0*	*82.0*	*20.7*	1888, 1809, 1973
WP2 pKM101	**MMS**	2.0 µL	*2587.0*	*23.6*	*12.1*	2570, 2577, 2614
WP2 uvrA pKM101	**MMS**	2.0 µL	*2307.0*	*46.6*	*6.0*	2262, 2304, 2355

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

TABLE 5 Pre-Experiment/Experiment I: 2166400 VV Plate Incorporation With Metabolic Activation

Study Name: 2166400
Experiment: 2166400 VV Plate
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 07.05.2021
Date Counted: 11.05.2021

With metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Propiconazole/**	3 µg	*16.3*	*1.2*	*1.1*	17, 17, 15
	Fenpropidin EC	10 µg	*14.0*	*2.0*	*1.0*	16, 12, 14
	(A9050B)	33 µg	*17.0*	*2.0*	*1.2*	19, 17, 15
		100 µg	*14.7*	*2.5*	*1.0*	12, 15, 17
		333 µg	*19.3*	*0.6*	*1.3*	19, 19, 20
		1000 µg	*14.3*	*2.1*	*1.0*	15 R, 12 R, 16 R
		2500 µg	*2.7*	*1.2*	*0.2*	4 P R, 2 P R, 2 P R
		5000 µg	*0.0*	*0.0*	*0.0*	0 P R, 0 P R, 0 P R
	Ethanol		*14.3*	*3.8*		10, 16, 17
	Untreated		*17.3*	*4.0*		15, 15, 22
TA 1537	**Propiconazole/**	3 µg	*12.0*	*4.4*	*1.1*	14, 15, 7
	Fenpropidin EC	10 µg	*12.7*	*4.0*	*1.2*	12, 17, 9
	(A9050B)	33 µg	*13.3*	*2.1*	*1.3*	11, 14, 15
		100 µg	*15.0*	*1.7*	*1.4*	14, 17, 14
		333 µg	*13.7*	*3.2*	*1.3*	15, 16, 10
		1000 µg	*7.7*	*2.1*	*0.7*	7, 6, 10
		2500 µg	*0.7*	*0.6*	*0.1*	1 P, 1 P, 0 P
		5000 µg	*0.3*	*0.6*	*0.0*	1 P, 0 P, 0 P
	Ethanol		*10.7*	*1.2*		10, 12, 10
	Untreated		*12.0*	*3.0*		9, 15, 12
TA 98	**Propiconazole/**	3 µg	*49.7*	*5.8*	*1.2*	53, 43, 53
	Fenpropidin EC	10 µg	*53.3*	*10.8*	*1.2*	41, 61, 58
	(A9050B)	33 µg	*50.3*	*5.1*	*1.2*	46, 56, 49
		100 µg	*40.7*	*3.5*	*0.9*	44, 41, 37
		333 µg	*49.3*	*8.5*	*1.1*	59, 43, 46
		1000 µg	*32.7*	*8.0*	*0.8*	41, 32, 25
		2500 µg	*3.3*	*1.2*	*0.1*	4 P, 4 P, 2 P
		5000 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*43.0*	*12.1*		57, 36, 36
	Untreated		*43.0*	*8.7*		38, 53, 38

Key to Plate Postfix Codes

P	Precipitate
R	Reduced background growth

Study Name: 2166400
Experiment: 2166400 VV Plate
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 07.05.2021
Date Counted: 11.05.2021

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Propiconazole/**	3 µg	*114.3*	*9.7*	*1.2*	112, 106, 125
	Fenpropidin EC	10 µg	*110.0*	*6.0*	*1.1*	116, 104, 110
	(A9050B)	33 µg	*124.7*	*4.7*	*1.3*	123, 121, 130
		100 µg	*115.3*	*10.1*	*1.2*	127, 109, 110
		333 µg	*86.0*	*3.5*	*0.9*	88, 82, 88
		1000 µg	*9.7*	*2.5*	*0.1*	12, 7, 10
		2500 µg	*0.3*	*0.6*	*0.0*	1 P, 0 P, 0 P
		5000 µg	*0.3*	*0.6*	*0.0*	1 P, 0 P, 0 P
	Ethanol		*96.0*	*3.0*		93, 96, 99
	Untreated		*113.7*	*12.7*		109, 104, 128
WP2	**Propiconazole/**	3 µg	*222.0*	*47.5*	*1.0*	273, 214, 179
pKM101	**Fenpropidin EC**	10 µg	*179.3*	*8.7*	*0.8*	172, 177, 189
	(A9050B)	33 µg	*195.7*	*8.7*	*0.9*	186, 203, 198
		100 µg	*163.3*	*14.5*	*0.7*	154, 156, 180
		333 µg	*132.3*	*7.1*	*0.6*	126, 140, 131
		1000 µg	*93.7*	*20.1*	*0.4*	88, 116, 77
		2500 µg	*13.0*	*2.6*	*0.1*	14 P, 15 P, 10 P
		5000 µg	*0.7*	*0.6*	*0.0*	0 P, 1 P, 1 P
	Ethanol		*220.0*	*18.1*		201, 237, 222
	Untreated		*241.3*	*17.0*		231, 232, 261
WP2	**Propiconazole/**	3 µg	*439.0*	*18.1*	*1.0*	456, 420, 441
uvrA	**Fenpropidin EC**	10 µg	*427.3*	*19.5*	*1.0*	427, 408, 447
pKM101	**(A9050B)**	33 µg	*428.7*	*26.2*	*1.0*	401, 453, 432
		100 µg	*402.7*	*17.5*	*0.9*	422, 388, 398
		333 µg	*314.0*	*45.6*	*0.7*	317, 358, 267
		1000 µg	*112.7*	*7.8*	*0.3*	119, 115, 104
		2500 µg	*101.7*	*7.0*	*0.2*	109 P, 101 P, 95 P
		5000 µg	*1.3*	*0.6*	*0.0*	2 P, 1 P, 1 P
	Ethanol		*430.0*	*6.6*		437, 429, 424
	Untreated		*413.3*	*38.5*		415, 451, 374
TA 1535	**2-AA**	2.5 µg	*251.3*	*20.6*	*17.5*	273, 232, 249
TA 1537	**2-AA**	2.5 µg	*397.7*	*19.5*	*37.3*	389, 384, 420
TA 98	**2-AA**	2.5 µg	*2371.7*	*175.0*	*55.2*	2306, 2570, 2239
TA 100	**2-AA**	2.5 µg	*3602.7*	*200.2*	*37.5*	3816, 3419, 3573
WP2 pKM101	**2-AA**	10.0 µg	*916.0*	*37.2*	*4.2*	929, 874, 945
WP2 uvrA pKM101	**2-AA**	10.0 µg	*1435.7*	*103.4*	*3.3*	1374, 1378, 1555

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate

TABLE 6 Experiment Ia: 2166400 VV Plate Incorporation Without Metabolic Activation

Study Name: 2166400
Experiment: 2166400 HV1a Plate
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 26.05.2021
Date Counted: 31.05.2021

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 pKM101	**Propiconazole/ Fenpropidin EC (A9050B)**	1 µg	*266.7*	*7.2*	*1.1*	263, 262, 275
		3 µg	*253.0*	*25.6*	*1.0*	277, 226, 256
		10 µg	*253.7*	*14.7*	*1.0*	265, 259, 237
		33 µg	*264.3*	*19.0*	*1.0*	245, 283, 265
		100 µg	*75.7*	*11.1*	*0.3*	86, 77, 64
		333 µg	*30.3*	*9.9*	*0.1*	37, 35, 19
		1000 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*252.0*	*18.2*		273, 241, 242
	Untreated		*290.0*	*32.9*		264, 279, 327
WP2 pKM101	**MMS**	2.0 µL	*3045.0*	*184.7*	*12.1*	2837, 3108, 3190

Key to Positive Controls

MMS methyl methane sulfonate

Key to Plate Postfix Codes

P Precipitate

TABLE 7 Experiment II: 2166400 HV2 Pre Incubation Without Metabolic Activation

Study Name: 2166400
Experiment: 2166400 HV2 Pre
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 26.05.2021
Date Counted: 31.05.2021

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Propiconazole/**	3 µg	*12.7*	*5.5*	*0.9*	9, 19, 10
	Fenpropidin EC	10 µg	*13.0*	*3.5*	*0.9*	15, 15, 9
	(A9050B)	33 µg	*15.3*	*4.7*	*1.0*	19, 17, 10
		100 µg	*8.0*	*2.6*	*0.5*	11, 7, 6
		333 µg	*8.7*	*2.3*	*0.6*	10, 10, 6
		1000 µg	*0.0*	*0.0*	*0.0*	0, 0, 0
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*14.7*	*4.6*		12, 12, 20
	Untreated		*14.0*	*2.0*		12, 14, 16
TA 1537	**Propiconazole/**	3 µg	*12.3*	*2.9*	*1.1*	14, 9, 14
	Fenpropidin EC	10 µg	*11.7*	*2.1*	*1.1*	14, 11, 10
	(A9050B)	33 µg	*13.3*	*2.3*	*1.2*	16, 12, 12
		100 µg	*13.7*	*2.5*	*1.2*	14, 11, 16
		333 µg	*7.0*	*2.6*	*0.6*	5, 10, 6
		1000 µg	*0.7*	*0.6*	*0.1*	1, 1, 0
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*11.0*	*2.6*		9, 10, 14
	Untreated		*11.3*	*2.5*		9, 14, 11
TA 98	**Propiconazole/**	3 µg	*34.3*	*7.8*	*0.9*	32, 43, 28
	Fenpropidin EC	10 µg	*25.3*	*6.8*	*0.7*	33, 23, 20
	(A9050B)	33 µg	*35.0*	*9.5*	*0.9*	25, 44, 36
		100 µg	*29.3*	*6.7*	*0.8*	31, 22, 35
		333 µg	*5.3*	*0.6*	*0.1*	5, 6, 5
		1000 µg	*0.3*	*0.6*	*0.0*	0, 0, 1
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*37.3*	*1.2*		38, 36, 38
	Untreated		*31.3*	*4.2*		36, 30, 28
TA 100	**Propiconazole/**	3 µg	*115.7*	*10.5*	*1.2*	126, 105, 116
	Fenpropidin EC	10 µg	*108.3*	*23.6*	*1.1*	135, 90, 100
	(A9050B)	33 µg	*95.7*	*3.1*	*1.0*	99, 95, 93
		100 µg	*69.0*	*7.8*	*0.7*	64, 78, 65
		333 µg	*28.0*	*7.0*	*0.3*	36, 23, 25
		1000 µg	*0.0*	*0.0*	*0.0*	0, 0, 0
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*98.3*	*6.8*		106, 96, 93
	Untreated		*107.7*	*10.7*		120, 101, 102

Key to Plate Postfix Codes

P Precipitate

Study Name: 2166400
Experiment: 2166400 HV2 Pre
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 26.05.2021
Date Counted: 31.05.2021

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2	**Propiconazole/**	1 µg	*283.3*	*14.2*	*1.1*	286, 296, 268
pKM101	**Fenpropidin EC**	3 µg	*247.3*	*10.7*	*0.9*	253, 254, 235
	(A9050B)	10 µg	*270.3*	*16.9*	*1.0*	278, 251, 282
		33 µg	*273.7*	*12.9*	*1.0*	259, 279, 283
		100 µg	*89.3*	*6.1*	*0.3*	88, 84, 96
		333 µg	*0.3*	*0.6*	*0.0*	0, 0, 1
		1000 µg	*0.0*	*0.0*	*0.0*	0, 0, 0
	Ethanol	2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Untreated		*262.0*	*11.5*		251, 274, 261
	Propiconazole/		*300.3*	*8.4*		310, 295, 296
WP2	**Propiconazole/**	3 µg	*340.3*	*24.1*	*1.0*	343, 363, 315
uvrA	**Fenpropidin EC**	10 µg	*327.3*	*15.8*	*1.0*	331, 341, 310
pKM101	**(A9050B)**	33 µg	*354.0*	*1.7*	*1.0*	353, 353, 356
		100 µg	*269.3*	*20.5*	*0.8*	249, 290, 269
		333 µg	*215.3*	*12.1*	*0.6*	228, 214, 204
		1000 µg	*3.3*	*1.2*	*0.0*	4, 4, 2
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*339.3*	*21.2*		363, 322, 333
	Untreated		*367.7*	*28.0*		389, 378, 336
TA 1535	**NaN3**	10 µg	*1161.3*	*89.8*	*79.2*	1221, 1205, 1058
TA 1537	**4-NOPD**	50 µg	*67.7*	*10.0*	*6.2*	78, 58, 67
TA 98	**4-NOPD**	10 µg	*363.0*	*21.7*	*9.7*	366, 340, 383
TA 100	**NaN3**	10 µg	*1771.0*	*51.1*	*18.0*	1826, 1762, 1725
WP2 pKM101	**MMS**	2.0 µL	*2734.7*	*64.3*	*10.4*	2723, 2677, 2804
WP2 uvrA pKM101	**MMS**	2.0 µL	*2332.0*	*81.3*	*6.9*	2245, 2345, 2406

Key to Positive Controls		Key to Plate Postfix Codes	
NaN3	sodium azide	P	Precipitate
4-NOPD	4-nitro-o-phenylene-diamine		
MMS	methyl methane sulfonate		

TABLE 8 Experiment II: 2166400 HV2 Pre Incubation With Metabolic Activation

Study Name: 2166400
Experiment: 2166400 HV2 Pre
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 26.05.2021
Date Counted: 31.05.2021

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Propiconazole/**	3 µg	*16.7*	*4.6*	*1.0*	14, 22, 14
	Fenpropidin EC	10 µg	*13.7*	*4.0*	*0.8*	16, 9, 16
	(A9050B)	33 µg	*15.0*	*4.0*	*0.9*	19, 11, 15
		100 µg	*9.7*	*2.5*	*0.6*	10, 12, 7
		333 µg	*10.3*	*3.5*	*0.6*	7, 14, 10
		1000 µg	*6.0*	*1.7*	*0.4*	4 P, 7 P, 7 P
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*16.7*	*3.1*		16, 14, 20
	Untreated		*11.7*	*2.5*		9, 14, 12
TA 1537	**Propiconazole/**	3 µg	*11.7*	*2.1*	*0.8*	14, 10, 11
	Fenpropidin EC	10 µg	*12.7*	*5.5*	*0.8*	9, 19, 10
	(A9050B)	33 µg	*13.3*	*5.1*	*0.9*	12, 19, 9
		100 µg	*13.7*	*4.0*	*0.9*	16, 9, 16
		333 µg	*12.0*	*1.7*	*0.8*	14, 11, 11
		1000 µg	*1.7*	*0.6*	*0.1*	2 P, 2 P, 1 P
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*15.3*	*1.5*		14, 17, 15
	Untreated		*14.0*	*4.4*		11, 12, 19
TA 98	**Propiconazole/**	3 µg	*52.7*	*8.5*	*1.0*	53, 44, 61
	Fenpropidin EC	10 µg	*48.3*	*5.5*	*1.0*	51, 42, 52
	(A9050B)	33 µg	*45.3*	*7.6*	*0.9*	47, 37, 52
		100 µg	*45.0*	*9.8*	*0.9*	37, 42, 56
		333 µg	*38.7*	*3.1*	*0.8*	42, 38, 36
		1000 µg	*0.7*	*0.6*	*0.0*	1 P, 1 P, 0 P
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*50.7*	*6.8*		43, 56, 53
	Untreated		*35.3*	*4.9*		41, 33, 32
TA 100	**Propiconazole/**	3 µg	*111.7*	*9.0*	*1.0*	122, 106, 107
	Fenpropidin EC	10 µg	*119.7*	*15.0*	*1.0*	110, 112, 137
	(A9050B)	33 µg	*119.7*	*12.7*	*1.0*	122, 131, 106
		100 µg	*89.7*	*11.1*	*0.8*	91, 78, 100
		333 µg	*15.0*	*2.6*	*0.1*	12, 16, 17
		1000 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
		2500 µg	*0.0*	*0.0*	*0.0*	0 P, 0 P, 0 P
	Ethanol		*117.3*	*6.4*		110, 120, 122
	Untreated		*108.7*	*4.7*		114, 107, 105

Key to Plate Postfix Codes

P Precipitate

Study Name: 2166400
Experiment: 2166400 HV2 Pre
Assay Conditions:

Study Code: ICCR 2166400
Date Plated: 26.05.2021
Date Counted: 31.05.2021

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2	**Propiconazole/**	1 µg	*276.3*	*13.3*	*1.1*	265, 291, 273
pKM101	**Fenpropidin EC**	3 µg	*274.3*	*9.3*	*1.0*	277, 282, 264
	(A9050B)	10 µg	*273.7*	*16.8*	*1.0*	293, 265, 263
		33 µg	*240.3*	*12.0*	*0.9*	241, 228, 252
		100 µg	*290.7*	*9.0*	*1.1*	300, 290, 282
		333 µg	*82.3*	*5.5*	*0.3*	88, 77, 82
		1000 µg	*49.3*	*7.6*	*0.2*	41 P, 56 P, 51 P
	Ethanol	2500 µg	*2.0*	*0.0*	*0.0*	2 P, 2 P, 2 P
	Untreated		*262.0*	*20.0*		245, 257, 284
	Propiconazole/		*337.3*	*13.9*		322, 349, 341
WP2	**Propiconazole/**	3 µg	*404.0*	*25.4*	*1.0*	415, 375, 422
uvrA	**Fenpropidin EC**	10 µg	*396.0*	*16.8*	*1.0*	415, 390, 383
pKM101	**(A9050B)**	33 µg	*408.0*	*19.3*	*1.0*	400, 430, 394
		100 µg	*435.7*	*20.1*	*1.1*	430, 458, 419
		333 µg	*196.0*	*20.9*	*0.5*	172, 206, 210
		1000 µg	*144.0*	*7.5*	*0.4*	143 P, 152 P, 137 P
		2500 µg	*30.7*	*11.6*	*0.1*	44 P, 23 P, 25 P
	Ethanol		*404.0*	*24.6*		425, 410, 377
	Untreated		*425.3*	*25.4*		440, 440, 396
TA 1535	**2-AA**	2.5 µg	*250.3*	*18.8*	*15.0*	238, 241, 272
TA 1537	**2-AA**	2.5 µg	*335.0*	*8.2*	*21.8*	326, 342, 337
TA 98	**2-AA**	2.5 µg	*2654.0*	*458.6*	*52.4*	2168, 2715, 3079
TA 100	**2-AA**	2.5 µg	*3824.7*	*381.0*	*32.6*	3826, 4205, 3443
WP2 pKM101	**2-AA**	10.0 µg	*973.0*	*47.1*	*3.7*	1018, 977, 924
WP2 uvrA pKM101	**2-AA**	10.0 µg	*1414.7*	*56.0*	*3.5*	1350, 1449, 1445

Key to Positive Controls		Key to Plate Postfix Codes	
2-AA	2-aminoanthracene	P	Precipitate

APPENDICES SECTION

APPENDIX 1 Historical Control Data

These data represent the laboratory´s historical control data from July 2018 until July 2020 representing approx. 600 experiments (WP2 pKM101, WP2 uvrA pKM101 the historical data are based on approx. 80 experiments).

The positive controls that used to compile the historical positive control data correspond to the positive control substances described in Methods; section 3.2.2 (Positive control substances).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	12	2.6	7	22	13	2.5	7	24
	Untreated control	12	2.9	6	26	13	2.8	7	23
	Positive control	1116	141.3	340	1612	346	72.1	170	736
TA1537	Solvent control	11	2.4	6	20	14	2.8	7	28
	Untreated control	11	2.8	5	22	14	3.2	7	30
	Positive control	83	22.1	48	400	286	98.7	82	630
TA 98	Solvent control	28	4.9	13	46	38	6.4	12	62
	Untreated control	29	5.0	14	48	41	6.8	14	64
	Positive control	421	91.2	216	1218	3275	774.9	322	5699
TA 100	Solvent control	127	30.7	63	214	131	30.0	72	214
	Untreated control	135	35.7	64	233	140	34.4	68	217
	Positive control	1759	273.4	511	2588	3566	837.6	553	5444
WP2 pKM 101	Solvent control	248	31.7	171	299	266	33.0	205	315
	Untreated control	269	26.6	212	346	299	28.2	233	345
	Positive control	3343	428.4	2332	4653	1092	257.8	933	2781
WP2uvrA pKM 101	Solvent control	322	31.6	248	388	375	38.5	287	466
	Untreated control	346	28.2	279	403	393	32.6	313	480
	Positive control	3176	468.5	2021	4717	1897	183.2	1270	2464

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

ICCR-Roßdorf GmbH
Institute for Competent Contract Research
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
8 Analytische Prüfungen an biologischen Materialien	**8** Analytical and clinical chemistry testing

22.11.2018, 21.02.2019, 12. bis 14.03.2019
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

Brandt

Dr. Astrid Brandt, Referentin, Wiesbaden, den **23. Oktober 2019**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, KLIMASCHUTZ, LANDWIRTSCHAFT UND VERBRAUCHERSCHUTZ

Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Mainzer Straße 80, D 65189 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, Climate Protection, Agriculture and Consumer Protection; Department II 10; P.O. Box 31 09; 65189 Wiesbaden
Translation of seal inscription: Hessian Ministry for Environment, Climate Protection, Agriculture and Consumer Protection

APPENDIX 3 Certificate of S9

ICCR ROßDORF

CERTIFICATE

ICCR-Roßdorf S9 Preparation Lot No. 291020D

Date of preparation: October 29, 2020
Recertification date: May 10, 2021

Protein assay: 31.2 mg protein / ml S9

Sterility: 0 colonies / ml S9 on glucose-minimal-agar

Salmonella typhimurium assay (AMES-test)

Treatment	µl S9 / plate	number of revertants in TA 98	number of revertants in TA 98 (Recertification)
negative	0	29	30
control	100	34	34
10 µg/plate 2-Aminoanthracene	0	84	86
	100	2898	1978
10 µg/plate Benzo(a)pyrene	0	30	25
	100	118	106

The S9 was obtained from the livers of male Wistar rats which received triple treatments of 80 mg / kg body weight Phenobarbital and ß-Naphthoflavone orally on consecutive days. The livers were prepared 24 hours after the last treatment.

S. Ebert Sabine Ebert — 20. MAI 2021
Quality Assurance Auditor, ICCR-Roßdorf GmbH — Date

20. MAI 2021
Dr. Steffen Naumann, Study Director, ICCR-Roßdorf GmbH — Date

ICCR-Roßdorf GmbH
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Geschäftsführer: Dr. Markus Schulz

SOP Origin TS-SOP S9_23

APPENDIX 4 Certificate of Analysis

Syngenta Crop Protection AG
GLP Testing Facility WMU
Analytical Development & Product Chemistry
Breitenloh 5
4333 Münchwilen, Switzerland

Certificate of Analysis

A9050B
propiconazole/fenpropidin
EC (125/275)
STH001-015-001

Batch Identification	**STH001-015-001**
Other Batch ID	1162447
Product Code	**A9050B**
Other Product Code(s)	CGA64250/CGA114900 EC (125/275)
Chemical Analysis (Active Ingredient content)	
– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of propiconazole (sum of CGA93590 and CGA93591)***	**13.1 % w/w corresponding to 125 g/l**
– **Content of CGA93590***	**7.49 % w/w corresponding to 71.4 g/l**
– **Content of CGA93591***	**5.61 % w/w corresponding to 53.5 g/l**
– **Content of fenpropidin***	**28.8 % w/w corresponding to 274 g/l**
	The Active Ingredient(s) content is within the FAO limits.
Methodology used for Characterization / Recertification	GC, oscillating density meter
Physical Analysis	
– **Appearance**	**light yellow liquid**
– **Density***	**953 kg/m^3**
Stability:	
– **Storage Temperature**	**< 30 °C**
– **Recertification Date**	**End of September 2025**

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 AG, Switzerland.

Study number of batch characterization: CHMU200985
Study number(s) of batch recertification: ---

Authorization: 12-Nov-2020

Urs Spuhler
Analytical Development & Product Chemistry