Propiconazole/Pydiflumetofen

Propiconazole/Pydiflumetofen SE (A21573C) - *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 In den Leppsteinswiesen 19 64380 Rossdorf, Germany
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SPONSOR(S):	Syngenta Ltd. Jealott's Hill International Research Centre Bracknell, Berkshire RG42 6EY, United Kingdom

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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

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Date: 26 February 2021

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QUALITY ASSURANCE STATEMENT

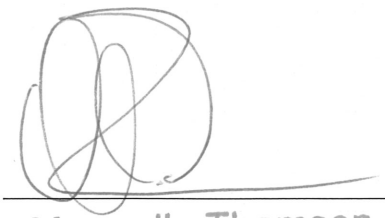
ICCR Study Number:	2131300
Test substance:	Propiconazole/Pydiflumetofen SE (A21573C)
Study director:	Dr. Steffi Chang
Study Title:	Propiconazole/Pydiflumetofen SE (A21573C) - *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay

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	23 October 2020	23 October 2020
Study Plan Amendment 1 Verification	11 November 2020	11 November 2020
Study Plan Amendment 2 Verification	18 November 2020	18 November 2020
Study Plan Amendment 3 Verification	22 February 2021	22 February 2021
Study Plan Amendment 4 Verification	25 February 2021	25 February 2021
Process – based Test System Preparation and Application	28 October 2020 12 November 2020	28 October 2020 12 November 2020
Report Audit	06 January 2021	06 January 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.



Manuella Thomsen

Quality Assurance Auditor
ICCR-Roßdorf GmbH

26 February 2021

Date

PROJECT STAFF SIGNATURE

Study Director — Dr. Steffi Chang

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Date: 26 February 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: 26 October 2020
Experimental start date: 30 October 2020
Experimental completion date: 07 December 2020

Deviations from the Guidelines
None

Retention of Samples
None

Performing Laboratory Test Substance Reference Number
S 2118811

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 item 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)

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

This study was performed to investigate the potential of Propiconazole/Pydiflumetofen SE (A21573C) to induce gene mutations in the plate incorporation test (Experiment I) and the pre-incubation test (Experiments II and IIa) 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).

Contamination was observed on the incubated agar plates in Experiment II in strain TA98 with and without S9 mix, this part of Experiment II was not evaluable and therefore repeated. The repeated experiment is reported as Experiment IIa.

1.2 Results

The plates incubated with the test item showed reduced background growth in all Salmonella typhimuruim strains in Experiment I, in all strains evaluated in Experiment II and in strain TA98 in the repeated Experiment IIa.

Cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in strains TA100 and WP2 *uvrA* (pKM101) in Experiment I, in all strains evaluated in Experiment II and in strain TA98 in the repeated Experiment IIa.

No relevant increase in revertant colony numbers of any of the six tester strains was observed following treatment with Propiconazole/Pydiflumetofen SE (A21573C) at any concentration level, 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/Pydiflumetofen SE (A21573C) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, Propiconazole/Pydiflumetofen SE (A21573C) 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 a negative result was obtained in this experiment, Experiments II and IIa were performed as pre-incubation assays.

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 six concentrations with adequately spaced intervals were tested. The maximum concentration was 5000 µg/plate.

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/Pydiflumetofen SE (A21573C)
Batch:	MHA0D01-FA1
Content of Pydiflumetofen: Content of Propiconazole:	13.9% w/w corresponding to 153 g/L 11.3% w/w corresponding to 125 g/L
Appearance:	off-white opaque, liquid
Recertification Date:	31 October 2023
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/Pydiflumetofen SE (A21573C).

Deionised water was initially selected as the solvent for the study. However, the sterile control using this solvent was found to be contaminated, therefore the experiment was repeated with THF as the solvent. On the day of the experiment (immediately before use), the test substance was suspended in tetrahydrofuran (THF, purity 99,85%). The solvent was chosen as the best suitable solvent compared to water, DMSO, DMF, and ethanol, according to its solubilisation and sterility 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.:	MKCG 1346
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 (Appendix 3).

The protein concentration in the S9 preparation was 34.8 mg/mL (lot no. 030920D) in all experiments.

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 initial pre-experiment using deionised water as the vehicle was rejected due to contamination being present in the sterility control. Therefore this was repeated using THF as the vehicle. The replacement 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. Since cytotoxic effects were observed in Experiment I a minimum of six concentrations were tested in Experiment II. 5000 µg/plate was chosen as the maximal concentration in Experiment II.

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

Strains TA1535, TA1537 and TA98:	33; 100; 333; 1000; 2500; and 5000 µg/plate
Strains TA100, WP2 (pKM101), and WP2 *uvrA* (pKM101):	10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Contamination was observed in Experiment II in strain TA98 with and without S9 mix, therefore this part of Experiment II was not evaluable and therefore repeated (reported as Experiment IIa). Due to cytotoxicity observed in the remaining strains in Experiment II, seven concentrations were tested in Experiment IIa with the following concentrations:

10; 33; 100; 333; 1000; 2500; and 5000 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:

25 µL	Test solution at each concentration, solvent (negative control)
100 µL	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 (25 µL) (solvent) or reference mutagen solution (positive control) (100 µL), 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 (25 µL) 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 in mutant colony frequencies of at least 2-fold concurrent 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 twice or above 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/Pydiflumetofen SE (A21573C), was assessed for its potential to induce gene mutations in the plate incorporation test (Experiment I) and the pre-incubation test (Experiments II and IIa) using S. typhimurium strains TA1535, TA1537, TA98, and TA100, and the E. coli strains WP2 (pKM101) and WP2 uvrA (pKM101).

The initial pre-experiment was rejected due to contamination being present in the sterility control. In the repeat valid pre-experiment the concentration range of the test substance was 3 - 5000 µg/plate. This pre-experiment is reported as Experiment I. Since cytotoxic effects were observed in Experiment I a minimum of six concentrations were tested in Experiment II. 5000 µg/plate was chosen as the maximal concentration in Experiment II.

The assay was performed with and without liver microsomal activation. 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 II:	
Strains TA1535, TA1537 and TA98:	33; 100; 333; 1000; 2500; and 5000 µg/plate
Strains TA100, WP2 (pKM101), and WP2 *uvrA* (pKM101):	10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Contamination was observed in Experiment II in strain TA98 with and without S9 mix, therefore this part of Experiment II was not evaluable and was repeated (reported as Experiment IIa). Due to cytotoxicity observed in the remaining strains in Experiment II, seven concentrations were tested in Experiment IIa with the following concentrations:

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

The test item precipitated in the overlay agar in the test tubes from 2500 to 5000 µg/plate in all experiments. Precipitation of the test item in the overlay agar on the incubated agar plates was observed in Experiment I at 5000 µg/plate. The undissolved particles had no influence on the data recording, a manual count was performed where required. In Experiments II and IIa no precipitation of the test item occurred up to the highest investigated concentration.

The plates incubated with the test item showed reduced background growth at the following concentrations (µg/plate):

Strain	Experiment I		Experiment II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA1535	2500 – 5000	/	2500 – 5000	2500 – 5000
TA1537	2500 – 5000	2500 – 5000	2500 – 5000	2500 – 5000
TA98	5000	5000	n.a.	n.a.
TA100	1000 – 5000	2500 – 5000	1000 – 5000	1000 – 5000
WP2 (pKM101)	/	/	1000 – 5000	2500 – 5000
WP2 *uvrA* (pKM101)	/	/	1000 – 5000	5000

/ = normal background growth
n.a. = not analysable

Strain	Experiment IIa	
	without S9 mix	with S9 mix
TA1535	n.p.	n.p.
TA1537	n.p.	n.p.
TA98	2500 – 5000	2500 – 5000
TA100	n.p.	n.p.
WP2 (pKM101)	n.p.	n.p.
WP2 *uvrA* (pKM101)	n.p.	n.p.

n.p. = not performed

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 II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA1535	/	/	2500 – 5000	5000
TA1537	/	/	2500 – 5000	2500 – 5000
TA98	/	/	n.a.	n.a.
TA100	5000	5000	1000 – 5000	1000 – 5000
WP2 (pKM101)	/	/	1000 – 5000	5000
WP2 *uvrA* (pKM101)	5000	/	2500 – 5000	/

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

Strain	Experiment IIa	
	without S9 mix	with S9 mix
TA1535	n.p.	n.p.
TA1537	n.p.	n.p.
TA98	2500 – 5000	2500 – 5000
TA100	n.p.	n.p.
WP2 (pKM101)	n.p.	n.p.
WP2 *uvrA* (pKM101)	n.p.	n.p.

n.p. = not performed

No substantial increase in revertant colony numbers in any of the six tester strains was observed following treatment with Propiconazole/Pydiflumetofen SE (A21573C) at any concentration 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 in induced revertant colonies consistent with the laboratory's historical control data and 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.

5.0 CONCLUSIONS

In conclusion, it can be stated that during the described mutagenicity tests and under the experimental conditions reported, Propiconazole/Pydiflumetofen SE (A21573C) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, Propiconazole/Pydiflumetofen SE (A21573C) is considered to be non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

6.0 REFERENCES

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TABLES SECTION

TABLE 1 Summary of Results Pre-Experiment/Experiment I

Study Name: 2131300
Experiment: 2131300_VV_Plate
Assay Conditions:

Study Code: ICCR 2131300
Date Plated: 17.11.2020
Date Counted: 20.11.2020

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	THF		10 ± 1	9 ± 3	24 ± 2	115 ± 14	274 ± 56	321 ± 23
	Untreated		14 ± 3	10 ± 3	27 ± 5	119 ± 7	308 ± 38	366 ± 23
	Propiconazole/	3 µg	8 ± 2	9 ± 3	25 ± 3	131 ± 11	321 ± 23	349 ± 27
	Pydiflumetofen	10 µg	11 ± 2	10 ± 2	29 ± 2	127 ± 17	306 ± 46	369 ± 19
	SE (A21573C)	33 µg	7 ± 2	10 ± 1	30 ± 2	113 ± 13	305 ± 37	325 ± 37
		100 µg	12 ± 3	13 ± 3	28 ± 7	115 ± 11	313 ± 23	338 ± 31
		333 µg	10 ± 3	9 ± 3	26 ± 5	116 ± 18	290 ± 35	341 ± 29
		1000 µg	8 ± 2	10 ± 4	21 ± 2	87 ± 10 [R]	228 ± 40	323 ± 16
		2500 µg	7 ± 3 [MR]	10 ± 1 [R]	26 ± 7	76 ± 13 [R]	221 ± 25	286 ± 7
		5000 µg	8 ± 3 [PMR]	7 ± 2 [PMR]	26 ± 3 [PR]	6 ± 2 [PMR]	133 ± 18 [P]	134 ± 21 [P]
	NaN3	10 µg	1250 ± 46			1584 ± 80		
	4-NOPD	10 µg			403 ± 18			
	4-NOPD	50 µg		97 ± 14				
	MMS	2.0 µL					2856 ± 158	2589 ± 208
With Activation	THF		10 ± 1	14 ± 5	40 ± 11	129 ± 8	294 ± 47	340 ± 21
	Untreated		12 ± 3	11 ± 2	44 ± 4	147 ± 2	317 ± 17	404 ± 33
	Propiconazole/	3 µg	10 ± 1	13 ± 3	38 ± 8	143 ± 11	343 ± 15	412 ± 10
	Pydiflumetofen	10 µg	12 ± 2	13 ± 1	42 ± 8	154 ± 12	292 ± 20	397 ± 18
	SE (A21573C)	33 µg	12 ± 2	12 ± 2	42 ± 6	144 ± 9	313 ± 18	392 ± 24
		100 µg	12 ± 2	14 ± 1	40 ± 3	157 ± 8	301 ± 15	381 ± 24
		333 µg	11 ± 2	12 ± 1	45 ± 8	157 ± 16	283 ± 6	421 ± 29
		1000 µg	12 ± 4	10 ± 3	34 ± 4	132 ± 23	250 ± 27	390 ± 1
		2500 µg	7 ± 3	17 ± 3 [MR]	39 ± 2	97 ± 10 [R]	290 ± 36	381 ± 6
		5000 µg	9 ± 1 [PM]	19 ± 2 [PMR]	30 ± 7 [PR]	44 ± 4 [PMR]	169 ± 18 [P]	307 ± 42 [P]
	2-AA	2.5 µg	237 ± 11	399 ± 19	2997 ± 29	4155 ± 260		
	2-AA	10.0 µg					988 ± 12	1531 ± 59

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

R	Reduced background growth
P	Precipitate
M	Manual count

TABLE 2 Summary of Results Experiment II

Study Name: 2131300
Experiment: 2131300 HV2 Pre
Assay Conditions:

Study Code: ICCR 2131300
Date Plated: 26.11.2020
Date Counted: 30.11.2020

Metabolic Activation	Test Group	Concentration (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	THF		8 ± 2	11 ± 1	154 ± 11	211 ± 28	373 ± 12
	Untreated		9 ± 1	10 ± 1	182 ± 21	270 ± 19	386 ± 37
	Propiconazole/	10 µg			114 ± 7	183 ± 20	323 ± 2
	Pydiflumetofen	33 µg	9 ± 2	10 ± 1	124 ± 13	169 ± 16	334 ± 8
	SE (A21573C)	100 µg	8 ± 3	10 ± 3	89 ± 14	167 ± 33	305 ± 18
		333 µg	7 ± 2	15 ± 3	80 ± 7	129 ± 22	201 ± 22
		1000 µg	6 ± 1	7 ± 2	29 ± 5 M R	86 ± 8 M R	184 ± 20 R
		2500 µg	3 ± 0 M R	2 ± 1 M R	0 ± 0 R	10 ± 3 M R	1 ± 1 R
		5000 µg	3 ± 1 M R	0 ± 1 R	0 ± 0 R	3 ± 1 M R	1 ± 1 M R
	NaN3	10 µg	1167 ± 75		1738 ± 118		
	4-NOPD	10 µg					
	4-NOPD	50 µg		98 ± 11			
	MMS	2.0 µL				4280 ± 329	3923 ± 139
With Activation	THF		10 ± 4	17 ± 5	145 ± 7	310 ± 5	395 ± 31
	Untreated		10 ± 2	22 ± 5	142 ± 9	342 ± 13	392 ± 20
	Propiconazole/	10 µg			152 ± 15	313 ± 11	408 ± 22
	Pydiflumetofen	33 µg	11 ± 1	15 ± 1	162 ± 8	311 ± 21	375 ± 21
	SE (A21573C)	100 µg	11 ± 1	17 ± 3	148 ± 4	282 ± 3	397 ± 34
		333 µg	11 ± 2	18 ± 5	126 ± 14	313 ± 17	409 ± 6
		1000 µg	10 ± 3	15 ± 1	35 ± 7 M R	251 ± 48	342 ± 17
		2500 µg	5 ± 1 M R	6 ± 1 M R	20 ± 4 M R	152 ± 9 R	292 ± 17
		5000 µg	0 ± 0 M R	0 ± 0 R	4 ± 1 M R	38 ± 4 M R	201 ± 32 R
	2-AA	2.5 µg	209 ± 28	374 ± 5	3229 ± 191		
	2-AA	10.0 µg				949 ± 18	1948 ± 188

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

TABLE 3 Summary of Results Experiment IIa

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300 HV2a Pre Date Plated: 04.12.2020
Assay Conditions: Date Counted: 07.12.2020

Metabolic Activation	Test Group	Concentration (per plate)	Revertant Colony Counts (Mean ±SD)
			TA 98
Without Activation	THF		31 ± 10
	Untreated		27 ± 5
	Propiconazole/	10 µg	31 ± 9
	Pydiflumetofen	33 µg	25 ± 3
	SE (A21573C)	100 µg	25 ± 6
		333 µg	16 ± 4
		1000 µg	15 ± 4
		2500 µg	1 ± 1 [MR]
		5000 µg	0 ± 1 [MR]
	4-NOPD	10 µg	352 ± 28
With Activation	THF		47 ± 8
	Untreated		37 ± 6
	Propiconazole/	10 µg	37 ± 10
	Pydiflumetofen	33 µg	34 ± 4
	SE (A21573C)	100 µg	37 ± 3
		333 µg	34 ± 11
		1000 µg	24 ± 2
		2500 µg	4 ± 2 [MR]
		5000 µg	0 ± 1 [MR]
	2-AA	2.5 µg	3756 ± 403

Key to Positive Controls		Key to Plate Postfix Codes	
4-NOPD	4-nitro-o-phenylene-diamine	M	Manual count
2-AA	2-aminoanthracene	R	Reduced background growth

TABLE 4 Pre-Experiment and Experiment I: 2131300 VV Plate Incorporation Without Metabolic Activation

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300_VV_Plate Date Plated: 17.11.2020
Assay Conditions: Date Counted: 20.11.2020

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	*8.3*	*2.3*	*0.9*	7, 11, 7
	Pydiflumetofen	10 µg	*10.7*	*1.5*	*1.1*	9, 11, 12
	SE (A21573C)	33 µg	*7.0*	*1.7*	*0.7*	6, 6, 9
		100 µg	*11.7*	*2.5*	*1.2*	9, 12, 14
		333 µg	*10.0*	*3.5*	*1.0*	12, 12, 6
		1000 µg	*7.7*	*2.1*	*0.8*	6, 10, 7
		2500 µg	*6.7*	*2.9*	*0.7*	5 M R, 10 M R, 5 M R
		5000 µg	*8.0*	*3.5*	*0.8*	6 P M R, 12 P M R, 6 P M R
	THF		*9.7*	*1.2*		11, 9, 9
	Untreated		*13.7*	*2.9*		12, 17, 12
TA 1537	**Propiconazole/**	3 µg	*9.0*	*2.6*	*1.0*	11, 10, 6
	Pydiflumetofen	10 µg	*10.3*	*1.5*	*1.2*	9, 12, 10
	SE (A21573C)	33 µg	*10.0*	*1.0*	*1.2*	9, 10, 11
		100 µg	*12.7*	*3.2*	*1.5*	9, 14, 15
		333 µg	*9.3*	*2.5*	*1.1*	9, 12, 7
		1000 µg	*10.3*	*3.5*	*1.2*	10, 14, 7
		2500 µg	*9.7*	*0.6*	*1.1*	9 R, 10 R, 10 R
		5000 µg	*7.0*	*1.7*	*0.8*	8 P M R, 5 P M R, 8 P M R
	THF		*8.7*	*2.9*		7, 12, 7
	Untreated		*10.0*	*2.6*		11, 7, 12
TA 98	**Propiconazole/**	3 µg	*25.0*	*2.6*	*1.0*	26, 27, 22
	Pydiflumetofen	10 µg	*29.0*	*1.7*	*1.2*	30, 30, 27
	SE (A21573C)	33 µg	*29.7*	*1.5*	*1.2*	31, 28, 30
		100 µg	*27.7*	*6.8*	*1.1*	30, 33, 20
		333 µg	*26.0*	*5.0*	*1.1*	26, 21, 31
		1000 µg	*20.7*	*2.1*	*0.8*	23, 20, 19
		2500 µg	*26.3*	*7.0*	*1.1*	33, 27, 19
		5000 µg	*26.0*	*2.6*	*1.1*	27 P R, 28 P R, 23 P R
	THF		*24.3*	*2.3*		23, 23, 27
	Untreated		*27.0*	*5.3*		33, 25, 23

Key to Plate Postfix Codes

R	Reduced background growth
P	Precipitate
M	Manual count

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300_VV_Plate Date Plated: 17.11.2020
Assay Conditions: Date Counted: 20.11.2020

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	*131.0*	*10.6*	*1.1*	143, 127, 123
	Pydiflumetofen	10 µg	*127.3*	*17.4*	*1.1*	147, 121, 114
	SE (A21573C)	33 µg	*113.3*	*13.3*	*1.0*	110, 102, 128
		100 µg	*115.0*	*11.0*	*1.0*	115, 126, 104
		333 µg	*116.3*	*17.9*	*1.0*	112, 101, 136
		1000 µg	*86.7*	*9.9*	*0.8*	82 R, 98 R, 80 R
		2500 µg	*75.7*	*12.5*	*0.7*	67 R, 90 R, 70 R
		5000 µg	*6.0*	*1.7*	*0.1*	8 P M R, 5 P M R, 5 P M R
	THF		*114.7*	*13.6*		110, 104, 130
	Untreated		*118.7*	*6.5*		119, 125, 112
WP2 pKM101	**Propiconazole/**	3 µg	*321.0*	*23.3*	*1.2*	317, 300, 346
	Pydiflumetofen	10 µg	*306.0*	*46.1*	*1.1*	328, 337, 253
	SE (A21573C)	33 µg	*304.7*	*36.9*	*1.1*	338, 265, 311
		100 µg	*313.0*	*23.1*	*1.1*	289, 335, 315
		333 µg	*290.0*	*35.4*	*1.1*	320, 299, 251
		1000 µg	*228.3*	*40.4*	*0.8*	238, 184, 263
		2500 µg	*221.0*	*24.6*	*0.8*	203, 249, 211
		5000 µg	*133.3*	*17.5*	*0.5*	133 P, 116 P, 151 P
	THF		*273.7*	*56.0*		247, 338, 236
	Untreated		*307.7*	*38.0*		307, 270, 346
WP2 uvrA pKM101	**Propiconazole/**	3 µg	*349.0*	*26.6*	*1.1*	377, 346, 324
	Pydiflumetofen	10 µg	*369.3*	*19.1*	*1.2*	387, 372, 349
	SE (A21573C)	33 µg	*325.3*	*36.5*	*1.0*	325, 362, 289
		100 µg	*338.0*	*30.5*	*1.1*	359, 352, 303
		333 µg	*341.3*	*28.5*	*1.1*	363, 352, 309
		1000 µg	*323.0*	*15.9*	*1.0*	341, 317, 311
		2500 µg	*286.0*	*6.6*	*0.9*	293, 280, 285
		5000 µg	*134.3*	*21.1*	*0.4*	146 P, 147 P, 110 P
	THF		*321.0*	*22.6*		295, 336, 332
	Untreated		*366.3*	*22.6*		345, 390, 364
TA 1535	**NaN3**	10 µg	*1250.3*	*45.8*	*129.3*	1287, 1199, 1265
TA 1537	**4-NOPD**	50 µg	*96.7*	*13.9*	*11.2*	112, 93, 85
TA 98	**4-NOPD**	10 µg	*403.3*	*17.8*	*16.6*	383, 416, 411
TA 100	**NaN3**	10 µg	*1584.3*	*80.0*	*13.8*	1497, 1602, 1654
WP2 pKM101	**MMS**	2.0 µL	*2856.0*	*158.0*	*10.4*	2972, 2920, 2676
WP2 uvrA pKM101	**MMS**	2.0 µL	*2588.7*	*208.5*	*8.1*	2714, 2704, 2348

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

TABLE 5 Pre-Experiment and Experiment I: 2131300 VV Plate Incorporation With Metabolic Activation

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300_VV_Plate Date Plated: 17.11.2020
Assay Conditions: Date Counted: 20.11.2020

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	*9.7*	*0.6*	*0.9*	10, 10, 9
	Pydiflumetofen	10 µg	*12.3*	*1.5*	*1.2*	11, 12, 14
	SE (A21573C)	33 µg	*11.7*	*2.1*	*1.1*	11, 14, 10
		100 µg	*11.7*	*2.1*	*1.1*	11, 10, 14
		333 µg	*10.7*	*1.5*	*1.0*	11, 12, 9
		1000 µg	*11.7*	*3.8*	*1.1*	10, 16, 9
		2500 µg	*7.3*	*2.5*	*0.7*	7, 10, 5
		5000 µg	*9.3*	*0.6*	*0.9*	9 P M, 10 P M, 9 P M
	THF		*10.3*	*0.6*		10, 10, 11
	Untreated		*12.3*	*2.5*		12, 15, 10
TA 1537	**Propiconazole/**	3 µg	*13.0*	*3.5*	*1.0*	15, 9, 15
	Pydiflumetofen	10 µg	*12.7*	*1.2*	*0.9*	12, 12, 14
	SE (A21573C)	33 µg	*12.0*	*2.0*	*0.9*	12, 14, 10
		100 µg	*14.3*	*0.6*	*1.0*	14, 14, 15
		333 µg	*11.7*	*0.6*	*0.9*	12, 11, 12
		1000 µg	*10.3*	*2.9*	*0.8*	12, 7, 12
		2500 µg	*17.0*	*2.6*	*1.2*	15 M R, 16 M R, 20 M R
		5000 µg	*18.7*	*2.3*	*1.4*	20 P M R, 16 P M R, 20 P M R
	THF		*13.7*	*4.6*		19, 11, 11
	Untreated		*11.0*	*1.7*		12, 12, 9
TA 98	**Propiconazole/**	3 µg	*38.0*	*7.5*	*0.9*	46, 37, 31
	Pydiflumetofen	10 µg	*41.7*	*8.1*	*1.0*	37, 51, 37
	SE (A21573C)	33 µg	*42.0*	*5.6*	*1.0*	47, 36, 43
		100 µg	*39.7*	*2.9*	*1.0*	38, 43, 38
		333 µg	*44.7*	*8.1*	*1.1*	40, 40, 54
		1000 µg	*34.3*	*4.0*	*0.9*	35, 30, 38
		2500 µg	*39.3*	*2.1*	*1.0*	37, 41, 40
		5000 µg	*30.3*	*6.8*	*0.8*	38 P R, 28 P R, 25 P R
	THF		*40.3*	*11.0*		33, 35, 53
	Untreated		*44.3*	*3.5*		41, 48, 44

Key to Plate Postfix Codes

R	Reduced background growth
P	Precipitate
M	Manual count

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300_VV_Plate Date Plated: 17.11.2020
Assay Conditions: Date Counted: 20.11.2020

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	*143.3*	*10.5*	*1.1*	154, 143, 133
	Pydiflumetofen	10 µg	*154.0*	*12.3*	*1.2*	163, 159, 140
	SE (A21573C)	33 µg	*143.7*	*8.6*	*1.1*	153, 142, 136
		100 µg	*157.0*	*8.0*	*1.2*	157, 165, 149
		333 µg	*156.7*	*15.9*	*1.2*	147, 148, 175
		1000 µg	*132.0*	*22.7*	*1.0*	158, 122, 116
		2500 µg	*97.0*	*10.1*	*0.8*	99 R, 86 R, 106 R
		5000 µg	*44.0*	*4.4*	*0.3*	46 P M R, 47 P M R, 39 P M R
	THF		*128.7*	*7.8*		135, 120, 131
	Untreated		*147.3*	*1.5*		146, 149, 147
WP2 pKM101	**Propiconazole/**	3 µg	*343.3*	*14.8*	*1.2*	356, 347, 327
	Pydiflumetofen	10 µg	*291.7*	*20.3*	*1.0*	315, 282, 278
	SE (A21573C)	33 µg	*313.3*	*17.7*	*1.1*	322, 293, 325
		100 µg	*300.7*	*15.3*	*1.0*	309, 283, 310
		333 µg	*283.3*	*5.8*	*1.0*	290, 280, 280
		1000 µg	*250.0*	*26.9*	*0.9*	264, 219, 267
		2500 µg	*290.0*	*36.0*	*1.0*	254, 326, 290
		5000 µg	*169.3*	*17.6*	*0.6*	188 P, 167 P, 153 P
	THF		*293.7*	*46.6*		273, 347, 261
	Untreated		*317.3*	*17.0*		330, 324, 298
WP2 uvrA pKM101	**Propiconazole/**	3 µg	*412.3*	*10.0*	*1.2*	401, 416, 420
	Pydiflumetofen	10 µg	*396.7*	*17.8*	*1.2*	417, 384, 389
	SE (A21573C)	33 µg	*392.3*	*23.7*	*1.2*	399, 412, 366
		100 µg	*381.3*	*23.7*	*1.1*	394, 396, 354
		333 µg	*421.3*	*29.1*	*1.2*	398, 454, 412
		1000 µg	*390.0*	*1.0*	*1.1*	390, 389, 391
		2500 µg	*380.7*	*5.8*	*1.1*	384, 384, 374
		5000 µg	*307.3*	*42.2*	*0.9*	356 P, 282 P, 284 P
	THF		*340.0*	*21.0*		340, 361, 319
	Untreated		*404.3*	*32.7*		387, 442, 384
TA 1535	**2-AA**	2.5 µg	*237.3*	*11.2*	*23.0*	240, 225, 247
TA 1537	**2-AA**	2.5 µg	*398.7*	*18.7*	*29.2*	385, 391, 420
TA 98	**2-AA**	2.5 µg	*2997.0*	*29.5*	*74.3*	2991, 3029, 2971
TA 100	**2-AA**	2.5 µg	*4154.7*	*259.7*	*32.3*	4313, 4296, 3855
WP2 pKM101	**2-AA**	10.0 µg	*988.0*	*12.1*	*3.4*	995, 974, 995
WP2 uvrA pKM101	**2-AA**	10.0 µg	*1530.7*	*58.7*	*4.5*	1463, 1567, 1562

Key to Positive Controls

2-AA	2-aminoanthracene

Key to Plate Postfix Codes

R	Reduced background growth
P	Precipitate
M	Manual count

TABLE 6 Experiment II: 2131300 HV2 Pre Incubation Without Metabolic Activation

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300 HV2 Pre Date Plated: 26.11.2020
Assay Conditions: Date Counted: 30.11.2020

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/**	33 µg	*8.7*	*1.5*	*1.0*	9, 7, 10
	Pydiflumetofen	100 µg	*8.3*	*2.9*	*1.0*	10, 10, 5
	SE (A21573C)	333 µg	*7.3*	*1.5*	*0.9*	9, 7, 6
		1000 µg	*6.0*	*1.0*	*0.7*	6, 5, 7
		2500 µg	*3.0*	*0.0*	*0.4*	3 M R, 3 M R, 3 M R
		5000 µg	*3.0*	*1.0*	*0.4*	3 M R, 2 M R, 4 M R
	THF		*8.3*	*2.1*		9, 6, 10
	Untreated		*9.3*	*0.6*		9, 9, 10
TA 1537	**Propiconazole/**	33 µg	*10.3*	*1.2*	*0.9*	11, 9, 11
	Pydiflumetofen	100 µg	*10.3*	*2.9*	*0.9*	12, 12, 7
	SE (A21573C)	333 µg	*14.7*	*3.2*	*1.3*	11, 17, 16
		1000 µg	*7.0*	*2.0*	*0.6*	5, 9, 7
		2500 µg	*2.3*	*0.6*	*0.2*	2 M R, 3 M R, 2 M R
		5000 µg	*0.3*	*0.6*	*0.0*	0 R, 0 R, 1 R
	THF		*11.0*	*1.0*		11, 10, 12
	Untreated		*10.3*	*1.2*		9, 11, 11
TA 100	**Propiconazole/**	10 µg	*114.0*	*6.6*	*0.7*	115, 120, 107
	Pydiflumetofen	33 µg	*124.3*	*13.1*	*0.8*	112, 123, 138
	SE (A21573C)	100 µg	*89.0*	*14.0*	*0.6*	105, 79, 83
		333 µg	*80.0*	*7.0*	*0.5*	75, 88, 77
		1000 µg	*29.0*	*5.0*	*0.2*	29 M R, 24 M R, 34 M R
		2500 µg	*0.0*	*0.0*	*0.0*	0 R, 0 R, 0 R
		5000 µg	*0.0*	*0.0*	*0.0*	0 R, 0 R, 0 R
	THF		*154.3*	*11.2*		157, 164, 142
	Untreated		*182.3*	*21.1*		194, 195, 158
WP2 pKM101	**Propiconazole/**	10 µg	*183.0*	*19.7*	*0.9*	186, 201, 162
	Pydiflumetofen	33 µg	*169.3*	*16.3*	*0.8*	175, 151, 182
	SE (A21573C)	100 µg	*167.3*	*33.0*	*0.8*	161, 203, 138
		333 µg	*128.7*	*21.6*	*0.6*	104, 144, 138
		1000 µg	*85.7*	*7.6*	*0.4*	89 M R, 77 M R, 91 M R
		2500 µg	*10.3*	*2.5*	*0.0*	10 M R, 8 M R, 13 M R
		5000 µg	*3.3*	*0.6*	*0.0*	3 M R, 4 M R, 3 M R
	THF		*211.3*	*28.1*		238, 214, 182
	Untreated		*270.3*	*18.5*		249, 282, 280

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300 HV2 Pre Date Plated: 26.11.2020
Assay Conditions: Date Counted: 30.11.2020

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2	**Propiconazole/**	10 µg	*323.0*	*1.7*	*0.9*	324, 324, 321
uvrA	**Pydiflumetofen**	33 µg	*333.7*	*7.6*	*0.9*	342, 332, 327
pKM101	**SE (A21573C)**	100 µg	*305.3*	*18.2*	*0.8*	311, 320, 285
		333 µg	*200.7*	*21.5*	*0.5*	201, 179, 222
		1000 µg	*183.7*	*20.0*	*0.5*	203 R, 185 R, 163 R
		2500 µg	*1.3*	*0.6*	*0.0*	2 R, 1 R, 1 R
		5000 µg	*1.3*	*0.6*	*0.0*	1 M R, 1 M R, 2 M R
	THF		*373.0*	*12.3*		378, 359, 382
	Untreated		*386.0*	*37.3*		367, 362, 429
TA 1535	**NaN3**	10 µg	*1167.3*	*75.3*	*140.1*	1248, 1155, 1099
TA 1537	**4-NOPD**	50 µg	*98.3*	*11.0*	*8.9*	91, 93, 111
TA 98	**4-NOPD**	10 µg				
TA 100	**NaN3**	10 µg	*1738.3*	*118.4*	*11.3*	1672, 1668, 1875
WP2 pKM101	**MMS**	2.0 µL	*4280.0*	*328.8*	*20.3*	4653, 4032, 4155
WP2 uvrA pKM101	**MMS**	2.0 µL	*3923.0*	*139.5*	*10.5*	4081, 3871, 3817

Key to Positive Controls	
NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes	
M	Manual count
R	Reduced background growth

TABLE 7 Experiment II: 2131300 HV2 Pre Incubation With Metabolic Activation

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300 HV2 Pre Date Plated: 26.11.2020
Assay Conditions: Date Counted: 30.11.2020

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/**	33 µg	*11.3*	*0.6*	*1.1*	12, 11, 11
	Pydiflumetofen	100 µg	*11.0*	*1.0*	*1.1*	12, 11, 10
	SE (A21573C)	333 µg	*11.0*	*1.7*	*1.1*	12, 9, 12
		1000 µg	*10.0*	*2.6*	*1.0*	11, 7, 12
		2500 µg	*4.7*	*0.6*	*0.5*	4 M R, 5 M R, 5 M R
		5000 µg	*0.0*	*0.0*	*0.0*	0 M R, 0 M R, 0 M R
	THF		*10.3*	*3.5*		14, 10, 7
	Untreated		*10.3*	*1.5*		9, 10, 12
TA 1537	**Propiconazole/**	33 µg	*14.7*	*0.6*	*0.9*	15, 14, 15
	Pydiflumetofen	100 µg	*17.3*	*2.5*	*1.0*	15, 17, 20
	SE (A21573C)	333 µg	*17.7*	*4.7*	*1.0*	14, 16, 23
		1000 µg	*14.7*	*0.6*	*0.9*	15, 14, 15
		2500 µg	*5.7*	*0.6*	*0.3*	5 M R, 6 M R, 6 M R
		5000 µg	*0.0*	*0.0*	*0.0*	0 R, 0 R, 0 R
	THF		*17.0*	*5.0*		17, 22, 12
	Untreated		*21.7*	*4.5*		26, 22, 17
TA 100	**Propiconazole/**	10 µg	*152.0*	*14.7*	*1.0*	144, 143, 169
	Pydiflumetofen	33 µg	*162.3*	*8.0*	*1.1*	163, 170, 154
	SE (A21573C)	100 µg	*147.7*	*4.0*	*1.0*	144, 147, 152
		333 µg	*125.7*	*14.5*	*0.9*	109, 135, 133
		1000 µg	*34.7*	*6.8*	*0.2*	40 M R, 37 M R, 27 M R
		2500 µg	*20.3*	*4.0*	*0.1*	21 M R, 16 M R, 24 M R
		5000 µg	*3.7*	*0.6*	*0.0*	4 M R, 3 M R, 4 M R
	THF		*145.3*	*7.0*		146, 138, 152
	Untreated		*142.0*	*8.7*		137, 152, 137
WP2 pKM101	**Propiconazole/**	10 µg	*312.7*	*10.6*	*1.0*	303, 324, 311
	Pydiflumetofen	33 µg	*310.7*	*21.4*	*1.0*	322, 324, 286
	SE (A21573C)	100 µg	*281.7*	*3.2*	*0.9*	283, 284, 278
		333 µg	*313.0*	*17.3*	*1.0*	309, 332, 298
		1000 µg	*250.7*	*47.8*	*0.8*	295, 257, 200
		2500 µg	*151.7*	*8.7*	*0.5*	142 R, 159 R, 154 R
		5000 µg	*38.0*	*3.6*	*0.1*	37 M R, 42 M R, 35 M R
	THF		*310.0*	*5.3*		314, 304, 312
	Untreated		*342.3*	*12.7*		356, 340, 331

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300 HV2 Pre Date Plated: 26.11.2020
Assay Conditions: Date Counted: 30.11.2020

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2	**Propiconazole/**	10 µg	*408.3*	*22.2*	*1.0*	405, 388, 432
uvrA	**Pydiflumetofen**	33 µg	*375.3*	*21.4*	*1.0*	380, 352, 394
pKM101	**SE (A21573C)**	100 µg	*397.3*	*34.3*	*1.0*	435, 368, 389
		333 µg	*409.0*	*6.0*	*1.0*	409, 403, 415
		1000 µg	*342.0*	*17.3*	*0.9*	361, 338, 327
		2500 µg	*291.7*	*17.2*	*0.7*	299, 272, 304
		5000 µg	*201.0*	*32.4*	*0.5*	224 R, 215 R, 164 R
	THF		*394.7*	*31.0*		425, 396, 363
	Untreated		*391.7*	*20.3*		415, 378, 382
TA 1535	**2-AA**	2.5 µg	*209.0*	*27.8*	*20.2*	241, 191, 195
TA 1537	**2-AA**	2.5 µg	*374.3*	*5.1*	*22.0*	373, 370, 380
TA 98	**2-AA**	2.5 µg				
TA 100	**2-AA**	2.5 µg	*3229.3*	*190.9*	*22.2*	3306, 3012, 3370
WP2 pKM101	**2-AA**	10.0 µg	*949.0*	*17.5*	*3.1*	948, 967, 932
WP2 uvrA pKM101	**2-AA**	10.0 µg	*1948.0*	*187.9*	*4.9*	1842, 1837, 2165

Key to Positive Controls		Key to Plate Postfix Codes	
2-AA	2-aminoanthracene	R	Reduced background growth

TABLE 8 Experiment IIa: 2131300 HV2a Pre Incubation Without Metabolic Activation

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300 HV2a Pre Date Plated: 04.12.2020
Assay Conditions: Date Counted: 07.12.2020

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 98	**Propiconazole/**	10 µg	*31.0*	*8.7*	*1.0*	36, 36, 21
	Pydiflumetofen	33 µg	*24.7*	*3.2*	*0.8*	26, 27, 21
	SE (A21573C)	100 µg	*25.3*	*5.9*	*0.8*	21, 32, 23
		333 µg	*16.0*	*4.0*	*0.5*	12, 16, 20
		1000 µg	*15.3*	*4.2*	*0.5*	20, 12, 14
		2500 µg	*1.3*	*0.6*	*0.0*	2 M R, 1 M R, 1 M R
		5000 µg	*0.3*	*0.6*	*0.0*	0 M R, 0 M R, 1 M R
	THF		*31.3*	*9.5*		41, 22, 31
	Untreated		*27.0*	*5.0*		32, 22, 27
TA 98	**4-NOPD**	10 µg	*352.0*	*27.7*	*11.2*	320, 369, 367

Key to Positive Controls

4-NOPD	4-nitro-o-phenylene-diamine

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth

TABLE 9 Experiment IIa: 2131300 HV2a Pre Incubation With Metabolic Activation

Study Name: 2131300 Study Code: ICCR 2131300
Experiment: 2131300 HV2a Pre Date Plated: 04.12.2020
Assay Conditions: Date Counted: 07.12.2020

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 98	**Propiconazole/**	10 µg	*36.7*	*9.8*	*0.8*	48, 31, 31
	Pydiflumetofen	33 µg	*34.0*	*3.6*	*0.7*	33, 31, 38
	SE (A21573C)	100 µg	*37.3*	*3.2*	*0.8*	35, 41, 36
		333 µg	*34.0*	*10.8*	*0.7*	25, 31, 46
		1000 µg	*23.7*	*2.1*	*0.5*	26, 22, 23
		2500 µg	*4.3*	*1.5*	*0.1*	3 M R, 4 M R, 6 M R
		5000 µg	*0.3*	*0.6*	*0.0*	0 M R, 1 M R, 0 M R
	THF		*47.3*	*8.1*		56, 46, 40
	Untreated		*36.7*	*5.7*		32, 43, 35
TA 98	**2-AA**	2.5 µg	*3755.7*	*402.6*	*79.3*	3976, 4000, 3291

Key to Positive Controls

2-AA	2-aminoanthracene

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth

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
	Solvent control	12	2.6	7	22	13	2.5	7	24
TA 1535	Untreated control	12	2.9	6	26	13	2.8	7	23
	Positive control	1116	141.3	340	1612	346	72.1	170	736
	Solvent control	11	2.4	6	20	14	2.8	7	28
TA1537	Untreated control	11	2.8	5	22	14	3.2	7	30
	Positive control	83	22.1	48	400	286	98.7	82	630
	Solvent control	28	4.9	13	46	38	6.4	12	62
TA 98	Untreated control	29	5.0	14	48	41	6.8	14	64
	Positive control	421	91.2	216	1218	3275	774.9	322	5699
	Solvent control	127	30.7	63	214	131	30.0	72	214
TA 100	Untreated control	135	35.7	64	233	140	34.4	68	217
	Positive control	1759	273.4	511	2588	3566	837.6	553	5444
	Solvent control	248	31.7	171	299	266	33.0	205	315
WP2	Untreated control	269	26.6	212	346	299	28.2	233	345
pKM 101	Positive control	3343	428.4	2332	4653	1092	257.8	933	2781
	Solvent control	322	31.6	248	388	375	38.5	287	466
WP2uvrA	Untreated control	346	28.2	279	403	393	32.6	313	480
pKM 101	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. 030920D

Date of preparation: September 03, 2020
Release date: November 11, 2020

Protein assay: 34.8 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
negative	0	27
control	100	34
10 µg/plate	0	87
2-Aminoanthracene	50	1732
10 µg/plate	0	29
Benzo(a)pyrene	100	97

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.

H. Pilawa — 17. NOV. 2020

Quality Assurance Auditor
ICCR-Roßdorf GmbH — Date

18. NOV. 2020

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

Syngenta Crop Protection, LLC
Analytical and Product Chemistry
Greensboro, NC 27409

Certificate of Analysis

A21573C

Batch ID 1157060 (MHA0D01-FA1)

Test Substance Name:	CGA64250/SYN545974 SE (125/150)
Common Name:	Propiconazole/Pydiflumetofen SE (125/150)
Material ID:	A21573C
Batch ID:	1157060
Other ID:	MHA0D01-FA1
Source:	Syngenta Crop Protection LLC. (Omaha Plant), 4111 Gibson Road, Omaha, NE 68107, US

Chemical Analysis

AI	% w/w	g/L
Pydiflumetofen	13.9	153
Propiconazole	11.3	125

Identity of the Active Ingredients: Confirmed

Methodology Used for Characterization: LC, mass spectrometry, oscillating density meter.

The Active Ingredient(s) content is within the FAO limits.

Physical Analysis

Analyte	Value	Units
Density	1.103	g/cm3

Appearance: off-white opaque liquid

Storage Temperature: <30°C

Re-certification Date:End of Oct/2023

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

COA Number: USGR200374

Page 1 of 2

The stability of this test substance will be determined concurrently through reanalysis of material held in inventory under GLP conditions at Syngenta Crop Protection, LLC, Greensboro, NC.

This Certificate of Analysis is summarizing data from a study that has been performed in compliance with Good Laboratory Practices per 40 CFR Part 160. Raw data, documentation, protocols, any amendments to study protocols and reports pertaining to this study are maintained in the Syngenta Crop Protection Archives in Greensboro, NC.

Study Number: USGR200374

Authorization: Sherry Perine

Sherry C Perine

Sherry Perine
Analytical and Product Chemistry Department

Oct. 8, 2020

Date