Ametryn/Bicyclopyrone

Ametryn/Bicyclopyrone SC (A16361B) - *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay

Final Report

TEST GUIDELINE(S): OECD 471 (2020)

AUTHOR(S): Dr. Steffi Chang

COMPLETION DATE: 22 March 2021

PERFORMING LABORATORY: ICCR-Roßdorf GmbH
In den Leppsteinswiesen 19
64380 Rossdorf, Germany

LABORATORY PROJECT ID: Report Number: 2148400
Study Number: 2148400
Task Number: TK0539251

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: 22 March 2021

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FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

ICCR Study Number: 2148400
Test substance: Ametryn/Bicyclopyrone SC (A16361B)
Study director: Dr. Steffi Chang
Study Title: Ametryn/Bicyclopyrone SC (A16361B) - *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	06 January 2021	06 January 2021
Process – based Assessment of Response Test item Preparation	 16 December 2020 05 + 08 January 2021	 16 December 2020 08 January 2021
Report Audit	25 February 2021	26 February 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.

H. Pilawa

22 March 2021

Date

Quality Assurance Auditor
ICCR-Roßdorf GmbH

PROJECT STAFF SIGNATURE

Study Director

Dr. Steffi Chang

S. C

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Date: 22 March 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
Carolina Vaccari	Syngenta Study Manager

Study Dates
Study initiation date: 11 January 2021
Experimental start date: 14 January 2021
Experimental completion date: 02 February 2021

Deviations from the Guidelines
None

Retention of Samples
None

Performing Laboratory Test Substance Reference Number
S 2130911

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)

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

1.1 Study Design

This study was performed to investigate the potential of Ametryn/Bicyclopyrone SC (A16361B) to induce gene mutations in the plate incorporation test (Experiment I) 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

The plates incubated with the test item showed normal background growth up to the maximal concentration of 5000 µg/plate with and without S9 mix in all strains used.

Cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in Experiment I in strains TA 1537 and WP2 (pKM101) in the presence and absence of S9 mix at 5000 µg/plate. In Experiment II no cytotoxic effects were observed.

No relevant increase in revertant colony numbers of any of the six tester strains was observed following treatment with Ametryn/Bicyclopyrone SC (A16361B) 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, Ametryn/Bicyclopyrone SC (A16361B) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, Ametryn/Bicyclopyrone SC (A16361B) 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, 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.

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:	Ametryn/Bicyclopyrone SC (A16361B)
Batch:	1149625 (GP200727)
Content of Ametryn: Content of Bicyclopyrone:	38.5% w/w corresponding to 432 g/L 5.09% w/w corresponding to 57.1 g/L
Appearance:	Off-white to tan, liquid
Recertification Date:	30 September 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 ametryn or bicyclopyrone.

On the day of the experiment (immediately before use), the test substance was suspended in deionised water. The solvent was chosen as the most suitable solvent compared to DMSO and ethanol, 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 (Appendix 3).

The protein concentration in the S9 preparation was 34.8 mg/mL (lot no. 030920D) in the pre-experiment / Experiment I and 30.0 mg/mL (lot no. 140520K, recertified 16 November 2020) in Experiment II.

3.4.2 S9 mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution. The amount of S9 supernatant was 10% v/v in the S9 mix. Cofactors 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. Since minor cytotoxic effects and precipitation of the test substance were observed in Experiment I, seven 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:

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:

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 = 1.1 - 1.2; wavelength = 500 nm; approx. $8x10^8$ cells/mL),
2000 µL	Overlay agar

For the pre-incubation method test solution (100 µL) (solvent or 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) 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 substance 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, Ametryn/Bicyclopyrone SC (A16361B), was assessed for its potential to induce gene mutations in the plate incorporation test (Experiment I) 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. Since minor cytotoxic effects and precipitation of the test substance were observed in Experiment I, seven 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:	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 both experiments. Precipitation of the test item in the overlay agar on the incubated agar plates was observed in Experiment I from 2500 to 5000 µg/plate. The undissolved particles had no influence on the data recording. In Experiment II no precipitation of the test item occurred up to the highest investigated dose.

The plates incubated with the test item showed normal background growth up to the maximal concentration of 5000 µg/plate with and without S9 mix in all strains used.

Cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in Experiment I in strains TA 1537 and WP2 (pKM101) in the presence and absence of S9 mix at 5000 µg/plate.
In Experiment II no cytotoxic effects were observed.

No relevant increase in revertant colony numbers of any of the six tester strains was observed following treatment with Ametryn/Bicyclopyrone SC (A16361B) 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. 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, Ametryn/Bicyclopyrone SC (A16361B) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, Ametryn/Bicyclopyrone SC (A16361B) is considered to be non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

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TABLES SECTION

TABLE 1 Summary of Results Pre-Experiment/Experiment I

Study Name: 2148400
Experiment: 2148400 VV Plate
Assay Conditions:

Study Code: ICCR 2148400
Date Plated: 14.01.2021
Date Counted: 21.01.2021

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	Deionised water		9 ± 3	12 ± 1	28 ± 2	143 ± 8	264 ± 25	346 ± 8
	Untreated		8 ± 3	13 ± 2	26 ± 10	137 ± 2	252 ± 10	341 ± 21
	Ametryn/	3 µg	13 ± 3	11 ± 3	30 ± 6	148 ± 12	258 ± 23	336 ± 28
	Bicyclopyrone SC	10 µg	11 ± 2	14 ± 3	25 ± 4	128 ± 8	265 ± 30	379 ± 22
	(A16361B)	33 µg	8 ± 3	10 ± 1	24 ± 7	128 ± 6	270 ± 13	339 ± 39
		100 µg	12 ± 3	10 ± 3	25 ± 4	123 ± 18	275 ± 8	349 ± 27
		333 µg	8 ± 2	9 ± 2	24 ± 5	115 ± 13	250 ± 11	320 ± 17
		1000 µg	9 ± 2	8 ± 2	27 ± 6	125 ± 6	200 ± 15	324 ± 14
		2500 µg	8 ± 3 P	6 ± 1 P	27 ± 5 P	135 ± 2 P	144 ± 20 P	305 ± 14 P
		5000 µg	8 ± 2 P M	5 ± 1 P M	18 ± 3 P M	93 ± 9 P M	101 ± 15 P M	191 ± 15 P M
	NaN3	10 µg	1162 ± 51			2016 ± 135		
	4-NOPD	10 µg			442 ± 17			
	4-NOPD	50 µg		72 ± 6				
	MMS	2.0 µL					3889 ± 149	3398 ± 215
With Activation	Deionised water		16 ± 1	13 ± 6	33 ± 6	129 ± 13	303 ± 13	344 ± 15
	Untreated		13 ± 3	13 ± 3	38 ± 3	131 ± 11	289 ± 9	339 ± 18
	Ametryn/	3 µg	16 ± 1	11 ± 4	36 ± 6	144 ± 11	295 ± 27	355 ± 17
	Bicyclopyrone SC	10 µg	13 ± 3	12 ± 2	33 ± 3	127 ± 8	301 ± 14	360 ± 11
	(A16361B)	33 µg	15 ± 5	13 ± 4	40 ± 9	144 ± 23	309 ± 39	405 ± 14
		100 µg	15 ± 5	13 ± 2	42 ± 8	124 ± 16	282 ± 4	397 ± 19
		333 µg	15 ± 2	11 ± 2	32 ± 4	137 ± 8	307 ± 18	370 ± 12
		1000 µg	11 ± 4	12 ± 3	32 ± 10	146 ± 13	263 ± 15	342 ± 12
		2500 µg	9 ± 3 P	6 ± 1 P	30 ± 3 P	148 ± 13 P	208 ± 16 P	341 ± 7 P
		5000 µg	8 ± 2 P M	6 ± 2 P M	17 ± 3 P M	110 ± 5 P M	103 ± 14 P M	209 ± 14 P M
	2-AA	2.5 µg	297 ± 25	421 ± 24	3499 ± 523	3609 ± 152		
	2-AA	10.0 µg					988 ± 33	1679 ± 55

Key to Positive Controls

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

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 2 Summary of Results Experiment II

Study Name: 2148400
Experiment: 2148400 HV2 Pre
Assay Conditions:

Study Code: ICCR 2148400
Date Plated: 27.01.2021
Date Counted: 02.02.2021

Metabolic Activation	Test Group	Concen-tration (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	Deionised water		12 ± 2	11 ± 3	26 ± 3	128 ± 17	249 ± 18	365 ± 36
	Untreated		14 ± 4	13 ± 3	28 ± 9	137 ± 10	256 ± 38	348 ± 39
	Ametryn/	10 µg	12 ± 3	12 ± 2	29 ± 3	134 ± 11	284 ± 22	386 ± 8
	Bicyclopyrone SC	33 µg	14 ± 2	14 ± 3	27 ± 6	118 ± 17	245 ± 40	329 ± 4
	(A16361B)	100 µg	10 ± 2	12 ± 4	32 ± 6	129 ± 6	246 ± 24	334 ± 24
		333 µg	11 ± 3	11 ± 1	35 ± 3	128 ± 7	264 ± 16	361 ± 16
		1000 µg	9 ± 3	9 ± 3	24 ± 6	137 ± 8	194 ± 27	353 ± 28
		2500 µg	11 ± 4	8 ± 3	24 ± 3	122 ± 9	187 ± 18	317 ± 14
		5000 µg	11 ± 3	7 ± 2	27 ± 6	144 ± 19	115 ± 7	284 ± 16
	NaN3	10 µg	1153 ± 69			1690 ± 35		
	4-NOPD	10 µg			371 ± 33			
	4-NOPD	50 µg		76 ± 13				
	MMS	2.0 µL					3120 ± 167	3066 ± 302
With Activation	Deionised water		9 ± 2	12 ± 3	37 ± 8	142 ± 5	298 ± 19	390 ± 1
	Untreated		11 ± 1	14 ± 3	39 ± 4	146 ± 10	293 ± 34	391 ± 12
	Ametryn/	10 µg	12 ± 3	14 ± 4	36 ± 4	168 ± 9	297 ± 14	418 ± 3
	Bicyclopyrone SC	33 µg	11 ± 3	16 ± 3	39 ± 9	165 ± 15	283 ± 18	395 ± 32
	(A16361B)	100 µg	12 ± 4	11 ± 1	46 ± 9	132 ± 7	280 ± 20	404 ± 12
		333 µg	9 ± 3	12 ± 4	36 ± 5	156 ± 16	294 ± 33	409 ± 25
		1000 µg	12 ± 1	14 ± 2	42 ± 11	148 ± 18	246 ± 32	407 ± 48
		2500 µg	14 ± 4	10 ± 2	31 ± 4	142 ± 27	201 ± 25	358 ± 34
		5000 µg	10 ± 1	8 ± 2	30 ± 3	132 ± 20	140 ± 5	395 ± 11
	2-AA	2.5 µg	322 ± 10	337 ± 42	3349 ± 384	4402 ± 385		
	2-AA	10.0 µg					994 ± 34	1687 ± 75

Key to Positive Controls

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

TABLE 3 Pre-Experiment and Experiment I: 2148400 VV Plate Incorporation Without Metabolic Activation

Study Name: 2148400 Study Code: ICCR 2148400
Experiment: 2148400 VV Plate Date Plated: 14.01.2021
Assay Conditions: Date Counted: 21.01.2021

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Ametryn/**	3 µg	*13.0*	*2.6*	*1.4*	10, 15, 14
	Bicyclopyrone	10 µg	*11.0*	*1.7*	*1.2*	12, 9, 12
	SC (A16361B)	33 µg	*8.0*	*2.6*	*0.9*	5, 9, 10
		100 µg	*12.0*	*3.0*	*1.3*	12, 15, 9
		333 µg	*7.7*	*2.1*	*0.8*	7, 6, 10
		1000 µg	*8.7*	*2.3*	*0.9*	10, 10, 6
		2500 µg	*8.3*	*3.2*	*0.9*	7 P, 12 P, 6 P
		5000 µg	*7.7*	*1.5*	*0.8*	6 P M, 9 P M, 8 P M
	Deionised water		*9.3*	*2.5*		7, 12, 9
	Untreated		*8.3*	*3.2*		12, 6, 7
TA 1537	**Ametryn/**	3 µg	*10.7*	*2.9*	*0.9*	9, 9, 14
	Bicyclopyrone	10 µg	*14.0*	*3.0*	*1.2*	17, 11, 14
	SC (A16361B)	33 µg	*9.7*	*0.6*	*0.8*	10, 10, 9
		100 µg	*10.0*	*2.6*	*0.9*	7, 11, 12
		333 µg	*9.3*	*2.1*	*0.8*	11, 10, 7
		1000 µg	*8.0*	*1.7*	*0.7*	7, 7, 10
		2500 µg	*6.0*	*1.0*	*0.5*	5 P, 6 P, 7 P
		5000 µg	*4.7*	*0.6*	*0.4*	4 P M, 5 P M, 5 P M
	Deionised water		*11.7*	*0.6*		12, 12, 11
	Untreated		*12.7*	*2.3*		14, 10, 14
TA 98	**Ametryn/**	3 µg	*30.0*	*6.1*	*1.1*	27, 37, 26
	Bicyclopyrone	10 µg	*24.7*	*4.2*	*0.9*	26, 28, 20
	SC (A16361B)	33 µg	*23.7*	*7.1*	*0.8*	30, 16, 25
		100 µg	*24.7*	*4.2*	*0.9*	20, 26, 28
		333 µg	*24.3*	*4.6*	*0.9*	27, 19, 27
		1000 µg	*26.7*	*5.5*	*1.0*	21, 27, 32
		2500 µg	*27.0*	*4.6*	*1.0*	31 P, 28 P, 22 P
		5000 µg	*17.7*	*2.5*	*0.6*	20 P M, 15 P M, 18 P M
	Deionised water		*28.0*	*1.7*		27, 30, 27
	Untreated		*25.7*	*9.9*		21, 37, 19

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

Study Name: 2148400 | Study Code: ICCR 2148400
Experiment: 2148400 VV Plate | Date Plated: 14.01.2021
Assay Conditions: | Date Counted: 21.01.2021

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Ametryn/**	3 µg	*148.0*	*12.1*	*1.0*	137, 161, 146
	Bicyclopyrone	10 µg	*127.7*	*7.6*	*0.9*	133, 119, 131
	SC (A16361B)	33 µg	*127.7*	*5.5*	*0.9*	128, 133, 122
		100 µg	*122.7*	*17.6*	*0.9*	141, 121, 106
		333 µg	*115.0*	*13.2*	*0.8*	100, 125, 120
		1000 µg	*125.0*	*6.1*	*0.9*	121, 132, 122
		2500 µg	*135.0*	*2.0*	*0.9*	133 P, 137 P, 135 P
		5000 µg	*92.7*	*9.5*	*0.6*	100 P M, 82 P M, 96 P M
	Deionised water		*143.0*	*7.5*		142, 136, 151
	Untreated		*137.0*	*1.7*		138, 135, 138
WP2 pKM101	**Ametryn/**	3 µg	*258.3*	*23.3*	*1.0*	285, 242, 248
	Bicyclopyrone	10 µg	*265.0*	*29.8*	*1.0*	273, 290, 232
	SC (A16361B)	33 µg	*270.0*	*13.1*	*1.0*	284, 258, 268
		100 µg	*275.3*	*7.6*	*1.0*	270, 284, 272
		333 µg	*250.0*	*10.6*	*0.9*	258, 254, 238
		1000 µg	*200.0*	*15.1*	*0.8*	216, 198, 186
		2500 µg	*144.0*	*20.1*	*0.5*	167 P, 135 P, 130 P
		5000 µg	*101.3*	*15.3*	*0.4*	118 P M, 98 P M, 88 P M
	Deionised water		*264.3*	*25.1*		246, 293, 254
	Untreated		*252.0*	*10.4*		257, 240, 259
WP2 uvrA pKM101	**Ametryn/**	3 µg	*336.0*	*28.2*	*1.0*	304, 347, 357
	Bicyclopyrone	10 µg	*379.0*	*21.9*	*1.1*	363, 370, 404
	SC (A16361B)	33 µg	*339.3*	*38.6*	*1.0*	310, 325, 383
		100 µg	*349.0*	*27.1*	*1.0*	337, 380, 330
		333 µg	*320.3*	*17.0*	*0.9*	338, 304, 319
		1000 µg	*323.7*	*13.7*	*0.9*	326, 336, 309
		2500 µg	*305.0*	*14.2*	*0.9*	321 P, 300 P, 294 P
		5000 µg	*191.3*	*15.0*	*0.6*	176 P M, 192 P M, 206 P M
	Deionised water		*346.3*	*8.0*		354, 347, 338
	Untreated		*341.0*	*20.5*		362, 340, 321
TA 1535	**NaN3**	10 µg	*1161.7*	*50.9*	*124.5*	1194, 1188, 1103
TA 1537	**4-NOPD**	50 µg	*72.3*	*5.5*	*6.2*	78, 67, 72
TA 98	**4-NOPD**	10 µg	*441.7*	*17.5*	*15.8*	461, 437, 427
TA 100	**NaN3**	10 µg	*2015.7*	*134.8*	*14.1*	1861, 2108, 2078
WP2 pKM101	**MMS**	2.0 µL	*3889.0*	*148.8*	*14.7*	3718, 3989, 3960
WP2 uvrA pKM101	**MMS**	2.0 µL	*3397.7*	*214.9*	*9.8*	3170, 3426, 3597

Key to Positive Controls

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

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

TABLE 4 Pre-Experiment and Experiment I: 2148400 VV Plate Incorporation With Metabolic Activation

Study Name: 2148400 Study Code: ICCR 2148400
Experiment: 2148400 VV Plate Date Plated: 14.01.2021
Assay Conditions: Date Counted: 21.01.2021

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Ametryn/**	3 µg	*16.3*	*0.6*	*1.0*	16, 17, 16
	Bicyclopyrone	10 µg	*12.7*	*3.1*	*0.8*	10, 16, 12
	SC (A16361B)	33 µg	*15.3*	*5.0*	*1.0*	16, 10, 20
		100 µg	*15.3*	*5.1*	*1.0*	11, 21, 14
		333 µg	*14.7*	*2.3*	*0.9*	12, 16, 16
		1000 µg	*11.3*	*4.0*	*0.7*	15, 7, 12
		2500 µg	*9.3*	*2.5*	*0.6*	9 P, 12 P, 7 P
		5000 µg	*7.7*	*1.5*	*0.5*	9 P M, 8 P M, 6 P M
	Deionised water		*16.0*	*1.0*		17, 15, 16
	Untreated		*13.3*	*3.1*		14, 10, 16
TA 1537	**Ametryn/**	3 µg	*11.3*	*4.0*	*0.9*	7, 12, 15
	Bicyclopyrone	10 µg	*12.0*	*2.0*	*0.9*	10, 12, 14
	SC (A16361B)	33 µg	*13.3*	*4.0*	*1.1*	9, 17, 14
		100 µg	*12.7*	*2.3*	*1.0*	14, 14, 10
		333 µg	*10.7*	*1.5*	*0.8*	12, 11, 9
		1000 µg	*12.3*	*2.9*	*1.0*	14, 14, 9
		2500 µg	*6.3*	*0.6*	*0.5*	6 P, 7 P, 6 P
		5000 µg	*5.7*	*1.5*	*0.4*	4 P M, 6 P M, 7 P M
	Deionised water		*12.7*	*5.5*		9, 19, 10
	Untreated		*13.0*	*3.5*		17, 11, 11
TA 98	**Ametryn/**	3 µg	*36.0*	*6.1*	*1.1*	33, 32, 43
	Bicyclopyrone	10 µg	*33.0*	*2.6*	*1.0*	36, 31, 32
	SC (A16361B)	33 µg	*40.0*	*9.0*	*1.2*	31, 40, 49
		100 µg	*42.0*	*8.2*	*1.3*	33, 49, 44
		333 µg	*32.0*	*3.6*	*1.0*	35, 33, 28
		1000 µg	*32.0*	*9.6*	*1.0*	43, 28, 25
		2500 µg	*29.7*	*3.2*	*0.9*	31 P, 32 P, 26 P
		5000 µg	*17.0*	*2.6*	*0.5*	20 P M, 16 P M, 15 P M
	Deionised water		*32.7*	*5.5*		27, 33, 38
	Untreated		*37.7*	*3.1*		35, 41, 37

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

Study Name: 2148400 Study Code: ICCR 2148400
Experiment: 2148400 VV Plate Date Plated: 14.01.2021
Assay Conditions: Date Counted: 21.01.2021

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Ametryn/**	3 µg	*143.7*	*10.7*	*1.1*	137, 156, 138
	Bicyclopyrone	10 µg	*127.3*	*8.0*	*1.0*	128, 135, 119
	SC (A16361B)	33 µg	*144.3*	*23.2*	*1.1*	141, 123, 169
		100 µg	*123.7*	*15.5*	*1.0*	111, 119, 141
		333 µg	*137.0*	*7.8*	*1.1*	132, 146, 133
		1000 µg	*146.3*	*12.7*	*1.1*	132, 156, 151
		2500 µg	*147.7*	*12.6*	*1.1*	161 P, 146 P, 136 P
		5000 µg	*110.0*	*5.3*	*0.9*	112 P M, 114 P M, 104 P M
	Deionised water		*129.3*	*12.6*		141, 116, 131
	Untreated		*131.0*	*10.6*		143, 123, 127
WP2 pKM101	**Ametryn/**	3 µg	*295.0*	*27.2*	*1.0*	288, 272, 325
	Bicyclopyrone	10 µg	*300.7*	*14.2*	*1.0*	294, 291, 317
	SC (A16361B)	33 µg	*309.0*	*39.4*	*1.0*	265, 341, 321
		100 µg	*281.7*	*4.2*	*0.9*	283, 277, 285
		333 µg	*307.0*	*17.6*	*1.0*	327, 294, 300
		1000 µg	*262.7*	*15.4*	*0.9*	245, 270, 273
		2500 µg	*208.0*	*15.7*	*0.7*	225 P, 205 P, 194 P
		5000 µg	*103.3*	*13.6*	*0.3*	114 P M, 108 P M, 88 P M
	Deionised water		*302.7*	*13.3*		288, 306, 314
	Untreated		*289.0*	*8.7*		294, 294, 279
WP2 uvrA pKM101	**Ametryn/**	3 µg	*354.7*	*16.9*	*1.0*	359, 369, 336
	Bicyclopyrone	10 µg	*359.7*	*10.7*	*1.0*	354, 353, 372
	SC (A16361B)	33 µg	*404.7*	*14.3*	*1.2*	408, 389, 417
		100 µg	*397.0*	*18.5*	*1.2*	396, 379, 416
		333 µg	*369.7*	*11.7*	*1.1*	380, 357, 372
		1000 µg	*341.7*	*11.5*	*1.0*	342, 330, 353
		2500 µg	*341.3*	*6.7*	*1.0*	337 P, 349 P, 338 P
		5000 µg	*209.3*	*14.0*	*0.6*	224 P M, 196 P M, 208 P M
	Deionised water		*344.3*	*15.3*		336, 362, 335
	Untreated		*339.3*	*17.6*		341, 321, 356
TA 1535	**2-AA**	2.5 µg	*297.0*	*25.0*	*18.6*	289, 277, 325
TA 1537	**2-AA**	2.5 µg	*421.3*	*24.2*	*33.3*	404, 449, 411
TA 98	**2-AA**	2.5 µg	*3498.7*	*523.4*	*107.1*	3581, 2939, 3976
TA 100	**2-AA**	2.5 µg	*3609.0*	*152.0*	*27.9*	3763, 3605, 3459
WP2 pKM101	**2-AA**	10.0 µg	*988.3*	*33.3*	*3.3*	950, 1005, 1010
WP2 uvrA pKM101	**2-AA**	10.0 µg	*1679.3*	*54.6*	*4.9*	1642, 1654, 1742

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate
M Manual count

TABLE 5 Experiment II: 2148400 HV2 Pre Incubation Without Metabolic Activation

Study Name: 2148400 Study Code: ICCR 2148400
Experiment: 2148400 HV2 Pre Date Plated: 27.01.2021
Assay Conditions: Date Counted: 02.02.2021

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Ametryn/**	10 µg	*12.3*	*2.9*	*1.0*	9, 14, 14
	Bicyclopyrone	33 µg	*13.7*	*2.3*	*1.1*	15, 11, 15
	SC (A16361B)	100 µg	*10.3*	*1.5*	*0.9*	9, 10, 12
		333 µg	*11.0*	*2.6*	*0.9*	10, 14, 9
		1000 µg	*8.7*	*2.9*	*0.7*	12, 7, 7
		2500 µg	*10.7*	*3.5*	*0.9*	14, 7, 11
		5000 µg	*11.3*	*2.5*	*0.9*	11, 9, 14
	Deionised water		*12.0*	*1.7*		11, 11, 14
	Untreated		*14.0*	*4.4*		11, 12, 19
TA 1537	**Ametryn/**	10 µg	*12.0*	*2.0*	*1.1*	10, 14, 12
	Bicyclopyrone	33 µg	*14.3*	*2.9*	*1.3*	16, 16, 11
	SC (A16361B)	100 µg	*12.3*	*4.2*	*1.1*	17, 11, 9
		333 µg	*11.3*	*0.6*	*1.0*	12, 11, 11
		1000 µg	*9.3*	*2.5*	*0.8*	7, 9, 12
		2500 µg	*8.0*	*2.6*	*0.7*	10, 5, 9
		5000 µg	*6.7*	*2.1*	*0.6*	6, 5, 9
	Deionised water		*11.3*	*2.5*		9, 11, 14
	Untreated		*12.7*	*3.2*		15, 9, 14
TA 98	**Ametryn/**	10 µg	*29.0*	*3.5*	*1.1*	27, 33, 27
	Bicyclopyrone	33 µg	*27.3*	*6.4*	*1.1*	31, 20, 31
	SC (A16361B)	100 µg	*31.7*	*5.5*	*1.2*	37, 26, 32
		333 µg	*35.0*	*2.6*	*1.4*	36, 32, 37
		1000 µg	*23.7*	*5.7*	*0.9*	19, 22, 30
		2500 µg	*24.0*	*2.6*	*0.9*	25, 21, 26
		5000 µg	*27.0*	*5.6*	*1.1*	32, 28, 21
	Deionised water		*25.7*	*2.5*		23, 26, 28
	Untreated		*28.0*	*8.7*		23, 38, 23
TA 100	**Ametryn/**	10 µg	*134.0*	*11.3*	*1.0*	141, 140, 121
	Bicyclopyrone	33 µg	*118.0*	*16.6*	*0.9*	125, 99, 130
	SC (A16361B)	100 µg	*128.7*	*5.9*	*1.0*	131, 122, 133
		333 µg	*128.0*	*7.0*	*1.0*	128, 121, 135
		1000 µg	*136.7*	*8.1*	*1.1*	132, 146, 132
		2500 µg	*121.7*	*9.3*	*1.0*	111, 126, 128
		5000 µg	*144.0*	*18.5*	*1.1*	151, 123, 158
	Deionised water		*128.0*	*17.1*		114, 147, 123
	Untreated		*136.7*	*10.2*		125, 141, 144

Study Name: 2148400 Study Code: ICCR 2148400
Experiment: 2148400 HV2 Pre Date Plated: 27.01.2021
Assay Conditions: Date Counted: 02.02.2021

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2	**Ametryn/**	10 µg	*284.3*	*21.6*	*1.1*	264, 307, 282
pKM101	**Bicyclopyrone**	33 µg	*244.7*	*39.5*	*1.0*	200, 275, 259
	SC (A16361B)	100 µg	*245.7*	*24.0*	*1.0*	221, 247, 269
		333 µg	*264.3*	*15.5*	*1.1*	253, 258, 282
		1000 µg	*194.3*	*26.6*	*0.8*	165, 217, 201
		2500 µg	*187.3*	*17.6*	*0.8*	207, 182, 173
		5000 µg	*115.3*	*7.2*	*0.5*	120, 107, 119
	Deionised water		*249.3*	*17.5*		249, 232, 267
	Untreated		*256.3*	*38.1*		259, 217, 293
WP2	**Ametryn/**	10 µg	*386.0*	*8.2*	*1.1*	384, 379, 395
uvrA	**Bicyclopyrone**	33 µg	*328.7*	*4.2*	*0.9*	330, 332, 324
pKM101	**SC (A16361B)**	100 µg	*333.7*	*23.7*	*0.9*	319, 321, 361
		333 µg	*360.7*	*15.9*	*1.0*	379, 352, 351
		1000 µg	*353.0*	*27.7*	*1.0*	368, 370, 321
		2500 µg	*317.0*	*14.0*	*0.9*	333, 307, 311
		5000 µg	*284.3*	*16.0*	*0.8*	283, 301, 269
	Deionised water		*364.7*	*36.5*		358, 404, 332
	Untreated		*348.3*	*39.0*		309, 349, 387
TA 1535	**NaN3**	10 µg	*1152.7*	*68.6*	*96.1*	1213, 1167, 1078
TA 1537	**4-NOPD**	50 µg	*76.0*	*13.0*	*6.7*	84, 61, 83
TA 98	**4-NOPD**	10 µg	*370.7*	*33.3*	*14.4*	349, 409, 354
TA 100	**NaN3**	10 µg	*1689.7*	*35.0*	*13.2*	1729, 1678, 1662
WP2 pKM101	**MMS**	2.0 µL	*3119.7*	*166.7*	*12.5*	3060, 2991, 3308
WP2 uvrA pKM101	**MMS**	2.0 µL	*3065.7*	*302.2*	*8.4*	2909, 2874, 3414

Key to Positive Controls

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

TABLE 6 Experiment II: 2148400 HV2 Pre Incubation With Metabolic Activation

Study Name: 2148400 Study Code: ICCR 2148400
Experiment: 2148400 HV2 Pre Date Plated: 27.01.2021
Assay Conditions: Date Counted: 02.02.2021

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Ametryn/**	10 µg	*12.3*	*3.2*	*1.4*	16, 11, 10
	Bicyclopyrone	33 µg	*11.0*	*3.5*	*1.2*	9, 9, 15
	SC (A16361B)	100 µg	*12.3*	*4.0*	*1.4*	10, 17, 10
		333 µg	*9.0*	*3.0*	*1.0*	9, 6, 12
		1000 µg	*11.7*	*0.6*	*1.3*	12, 12, 11
		2500 µg	*13.7*	*3.5*	*1.5*	14, 17, 10
		5000 µg	*9.7*	*0.6*	*1.1*	9, 10, 10
	Deionised water		*9.0*	*2.0*		7, 9, 11
	Untreated		*11.0*	*1.0*		12, 10, 11
TA 1537	**Ametryn/**	10 µg	*14.0*	*3.6*	*1.2*	17, 10, 15
	Bicyclopyrone	33 µg	*16.3*	*3.2*	*1.4*	20, 14, 15
	SC (A16361B)	100 µg	*11.0*	*1.0*	*0.9*	11, 12, 10
		333 µg	*12.3*	*4.2*	*1.0*	9, 11, 17
		1000 µg	*13.7*	*1.5*	*1.1*	14, 12, 15
		2500 µg	*9.7*	*2.3*	*0.8*	7, 11, 11
		5000 µg	*7.7*	*2.1*	*0.6*	10, 7, 6
	Deionised water		*12.0*	*2.6*		11, 10, 15
	Untreated		*14.3*	*2.9*		16, 16, 11
TA 98	**Ametryn/**	10 µg	*36.0*	*4.0*	*1.0*	32, 40, 36
	Bicyclopyrone	33 µg	*38.7*	*9.3*	*1.1*	31, 49, 36
	SC (A16361B)	100 µg	*46.3*	*8.5*	*1.3*	56, 40, 43
		333 µg	*35.7*	*4.5*	*1.0*	31, 40, 36
		1000 µg	*42.0*	*11.1*	*1.1*	40, 32, 54
		2500 µg	*31.3*	*4.0*	*0.9*	32, 27, 35
		5000 µg	*30.3*	*2.5*	*0.8*	30, 33, 28
	Deionised water		*36.7*	*7.6*		42, 40, 28
	Untreated		*39.3*	*3.8*		41, 35, 42
TA 100	**Ametryn/**	10 µg	*168.3*	*9.5*	*1.2*	161, 179, 165
	Bicyclopyrone	33 µg	*165.0*	*15.4*	*1.2*	178, 148, 169
	SC (A16361B)	100 µg	*132.0*	*6.9*	*0.9*	128, 128, 140
		333 µg	*156.0*	*16.1*	*1.1*	141, 154, 173
		1000 µg	*147.7*	*17.8*	*1.0*	144, 132, 167
		2500 µg	*142.3*	*26.6*	*1.0*	126, 128, 173
		5000 µg	*132.0*	*20.0*	*0.9*	127, 115, 154
	Deionised water		*141.7*	*5.1*		143, 146, 136
	Untreated		*146.0*	*9.8*		157, 143, 138

Study Name: 2148400 Study Code: ICCR 2148400
Experiment: 2148400 HV2 Pre Date Plated: 27.01.2021
Assay Conditions: Date Counted: 02.02.2021

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2	**Ametryn/**	10 µg	*296.7*	*13.6*	*1.0*	295, 311, 284
pKM101	**Bicyclopyrone**	33 µg	*283.3*	*18.3*	*1.0*	277, 304, 269
	SC (A16361B)	100 µg	*280.3*	*20.4*	*0.9*	295, 257, 289
		333 µg	*294.3*	*33.1*	*1.0*	306, 320, 257
		1000 µg	*245.7*	*32.3*	*0.8*	226, 228, 283
		2500 µg	*201.0*	*24.9*	*0.7*	228, 196, 179
		5000 µg	*139.7*	*4.5*	*0.5*	144, 140, 135
	Deionised water		*298.0*	*18.5*		319, 284, 291
	Untreated		*293.3*	*34.1*		312, 254, 314
WP2	**Ametryn/**	10 µg	*418.3*	*2.9*	*1.1*	420, 415, 420
uvrA	**Bicyclopyrone**	33 µg	*395.3*	*32.1*	*1.0*	426, 362, 398
pKM101	**SC (A16361B)**	100 µg	*403.7*	*11.7*	*1.0*	395, 417, 399
		333 µg	*409.3*	*25.1*	*1.1*	391, 438, 399
		1000 µg	*407.0*	*47.7*	*1.0*	352, 433, 436
		2500 µg	*357.7*	*33.6*	*0.9*	374, 380, 319
		5000 µg	*395.0*	*11.4*	*1.0*	403, 400, 382
	Deionised water		*389.7*	*1.2*		389, 389, 391
	Untreated		*390.7*	*12.4*		383, 384, 405
TA 1535	**2-AA**	2.5 µg	*321.7*	*9.9*	*35.7*	317, 315, 333
TA 1537	**2-AA**	2.5 µg	*337.0*	*42.3*	*28.1*	289, 369, 353
TA 98	**2-AA**	2.5 µg	*3349.0*	*383.5*	*91.3*	3348, 3733, 2966
TA 100	**2-AA**	2.5 µg	*4401.7*	*385.2*	*31.1*	4832, 4284, 4089
WP2 pKM101	**2-AA**	10.0 µg	*994.3*	*33.9*	*3.3*	1031, 964, 988
WP2 uvrA pKM101	**2-AA**	10.0 µg	*1687.0*	*74.6*	*4.3*	1768, 1672, 1621

Key to Positive Controls

2-AA	2-aminoanthracene

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.

17. NOV. 2020

Quality Assurance Auditor
ICCR-Roßdorf GmbH H. Pilawa — Date

18. NOV. 2020

Dr. Steffen Naumann
Study Director
ICCR-Roßdorf GmbH — Date

ICCR-Roßdorf GmbH
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Registergericht Darmstadt, HRB 6837, USt.-ID DE812333696
Geschäftsführer: Dr. Markus Schulz

SOP Origin TS-SOP S9_23

ICCR ROßDORF

CERTIFICATE

ICCR-Roßdorf S9 Preparation Lot No. 140520K

Date of preparation: May 14, 2020
Recertification date: November 16, 2020

Protein assay: 30 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	32	28
control	100	31	33
10 µg/plate	0	99	29
2-Aminoanthracene	100	2621	2232
2-Aminoanthracene	150	3541	2216
10 µg/plate	0	25	23
Benzo(a)pyrene	100	115	116

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.

Quality Assurance Auditor
ICCR-Roßdorf GmbH H. Pilawa

24. NOV. 2020
Date

Dr. Steffen Naumann
Study Director
ICCR-Roßdorf GmbH

25. NOV. 2020
Date

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Registergericht Darmstadt, HRB 6837, USt.-ID DE812333696
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

A16361B
Batch ID 1149625 (GP200727)

Test Substance Name:	G34162/NOA449280 SC (428.57/057.14)
Common Name:	Ametryn/Bicyclopyrone SC (428.57/057.14)
Material ID:	A16361B
Batch ID:	1149625
Other ID:	GP200727
Source:	Syngenta Crop Protection LLC., 410 Swing Road, Greensboro, NC 27409, US

Chemical Analysis

AI	% w/w	g/L
Bicyclopyrone	5.09	57.1
Ametryn	38.5	432

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	1.121	g/cm3

Appearance: off-white to tan liquid

Storage Temperature: <30°C

Re-certification Date:End of Sep/2023

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

COA Number: USGR200340

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: USGR200340

Authorization: William Meyerhoffer

W J Meyerhoffer — September 8, 2020

William Meyerhoffer — Date

Analytical and Product Chemistry Department