Thiamethoxam

Thiamethoxam SL (A23943A) - *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay

Final Report

TEST GUIDELINE(S):	OECD 471 (2020)
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SPONSOR(S):	Syngenta Ltd. Jealott's Hill International Research Centre Bracknell, Berkshire RG42 6EY, United Kingdom

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study performed in the test facility of ICCR-Roßdorf GmbH, In den Leppsteinswiesen 19, 64380 Rossdorf, Germany was conducted in compliance with Good Laboratory Practice Regulations:

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

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

EC Commission Directive 2004/10/EC

These procedures are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHW, MAFF, and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

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

Dr. Steffi Chang
Study Director Bacterial Systems

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Date

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FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

ICCR Study Number: 3977311
Test substance: Thiamethoxam SL (A23943A)
Study director: Dr. Steffi Chang
Study Title: Thiamethoxam SL (A23943A) - *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	16 December 2021	16 December 2021
Process – based Assessment of Response	13 January 2022	13 January 2022
Report Audit	22 March 2022	24 March 2022

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

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

S. Ebert
Sabine Ebert

Quality Assurance Auditor
ICCR-Roßdorf GmbH

09 June 2022
Date

PROJECT STAFF SIGNATURE

Study Director

Dr. Steffi Chang

.....

Date: 14 June 2022

GENERAL INFORMATION

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The following contributed to this report in the capacities indicated:

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Study Dates

Study initiation date:	17 December 2021
Experimental start date:	04 January 2022
Experimental completion date:	24 January 2022

Deviations from the Guidelines
None

Retention of Samples
None

Performing Laboratory Test Substance Reference Number
S 2205211

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

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

A sample of the test substance will not be archived.

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

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

Distribution of the report

Sponsor	2 electronic copies (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 thiamethoxam SL (A23943A) 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 substance showed normal background growth up to the maximal concentration of 5000 µg/plate with and without S9 mix in all strains used.

No cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in all strains with and without metabolic activation.

No relevant increase in revertant colony numbers of any of the six tester strains was observed following treatment with thiamethoxam SL (A23943A) 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, thiamethoxam SL (A23943A) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, thiamethoxam SL (A23943A) is considered to be negative (i.e. 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 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:	Thiamethoxam SL (A23943A)
Batch:	NSI001-085-017
Content of Thiamethoxam:	6.63% w/w corresponding to 75.42 g/L
Appearance:	Light yellow*, liquid
Recertification Date:	10 December 2023
Storage Conditions:	At room temperature
Stability in Solvent:	Not indicated by the Sponsor

* determined by ICCR Roßdorf GmbH staff

The test substance concentrations were not adjusted for the content of Thiamethoxam SL (A23943A).

On the day of the experiment (immediately before use), the test substance was dissolved 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:	Sigma-Aldrich, 82024 Taufkirchen, Germany
Batch No.:	STBJ7813
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 29.6 mg/mL (lot no. 080721D) in both 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 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 no cytotoxic effects were observed 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:

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 = 1.0 - 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 item some test groups were scored manually (as indicated on data tables).

3.8.2 Acceptability of the assay

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

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of the historical data
- the positive control substances should produce an increase above the threshold of twofold (strains TA 98, TA 100, WP2 uvrA (pKM101, and WP2 (pKM101))) or threefold (strains TA 1535 and TA 1537) the revertant colony count of the corresponding solvent control;
- a minimum of five analysable concentrations should be present with at least four showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5.

3.8.3 Evaluation of results

A test substance is considered as a mutagen if a biologically relevant increase in the number of revertants of twofold or above (strains TA 98, TA 100, WP2 uvrA (pKM101), and WP2 (pKM101)) or of threefold or above (strains TA 1535 and TA 1537) the spontaneous mutation rate of the corresponding solvent control is observed (1).

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

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

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

3.8.4 Biometry

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

3.9 Major Computerized System

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

4.0 RESULTS AND DISCUSSION

The test substance, thiamethoxam SL (A23943A), 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 no cytotoxic effects were observed 5000 µg/plate was chosen as the maximal concentration in Experiment II. This is the maximum concentration recommended in the OECD test guideline.

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

No precipitation of the test substance occurred in the overlay agar in the test tubes. Precipitation of the test item in the overlay agar on the incubated agar plates was observed at 5000 µg/plate in Experiment I only.

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

No cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers in any of the six tester strains was observed following treatment with thiamethoxam SL (A23943A) at any concentration, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

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

In experiment II, the data in solvent control of strain WP2 (pKM101) without S9 mix were slightly above our historical control data range. Since this deviation is rather small (300 versus 299 colonies), this effect is considered to be based upon biologically irrelevant fluctuations in the number of colonies.

5.0 CONCLUSIONS

In conclusion, it can be stated that during the described mutagenicity tests and under the experimental conditions reported, thiamethoxam SL (A23943A) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, thiamethoxam SL (A23943A) 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: 3977311
Experiment: 3977311 VV Plate
Assay Conditions:

Study Code: ICCR 3977311
Date Plated: 04.01.2022
Date Counted: 10.01.2022

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		14 ± 2	16 ± 2	24 ± 6	118 ± 20	298 ± 9	379 ± 20
	Untreated		15 ± 2	12 ± 3	29 ± 7	125 ± 3	337 ± 11	395 ± 5
	Thiamethoxam	3 µg	13 ± 4	12 ± 2	26 ± 9	119 ± 13	305 ± 6	399 ± 4
	SL (A23943A)	10 µg	11 ± 1	13 ± 3	31 ± 8	121 ± 11	319 ± 6	394 ± 6
		33 µg	15 ± 3	10 ± 3	28 ± 8	113 ± 2	304 ± 6	395 ± 5
		100 µg	13 ± 4	10 ± 4	27 ± 6	108 ± 10	313 ± 16	403 ± 12
		333 µg	12 ± 3	14 ± 4	32 ± 8	120 ± 10	318 ± 8	400 ± 4
		1000 µg	9 ± 3	10 ± 4	34 ± 9	135 ± 14	307 ± 2	401 ± 10
		2500 µg	9 ± 2	16 ± 1	29 ± 7	155 ± 14	309 ± 5	406 ± 6
		5000 µg	14 ± 2 P	15 ± 3 P	26 ± 1 P	141 ± 16 P	291 ± 35 P	322 ± 24 P
	NaN3	10 µg	929 ± 276			1509 ± 261		
	4-NOPD	10 µg			505 ± 36			
	4-NOPD	50 µg		85 ± 4				
	MMS	2.0 µL					3154 ± 164	3533 ± 277
With Activation	Deionised water		12 ± 3	15 ± 3	37 ± 10	117 ± 10	312 ± 3	408 ± 17
	Untreated		11 ± 1	17 ± 3	53 ± 8	110 ± 14	343 ± 3	470 ± 7
	Thiamethoxam	3 µg	12 ± 2	16 ± 3	50 ± 4	107 ± 12	328 ± 22	416 ± 18
	SL (A23943A)	10 µg	10 ± 3	14 ± 3	43 ± 7	112 ± 16	325 ± 16	416 ± 5
		33 µg	11 ± 1	15 ± 1	44 ± 12	119 ± 7	312 ± 13	423 ± 8
		100 µg	12 ± 2	15 ± 5	41 ± 9	103 ± 6	314 ± 15	418 ± 7
		333 µg	14 ± 4	18 ± 3	45 ± 9	110 ± 14	320 ± 10	404 ± 4
		1000 µg	19 ± 4	20 ± 4	47 ± 8	148 ± 11	316 ± 11	409 ± 11
		2500 µg	13 ± 1	16 ± 1	45 ± 7	153 ± 17	319 ± 5	398 ± 5
		5000 µg	12 ± 4 P	17 ± 2 P M	48 ± 11 P	150 ± 6 P	312 ± 12 P	385 ± 16 P
	2-AA	2.5 µg	311 ± 31	428 ± 35	2982 ± 326	3785 ± 191		
	2-AA	10.0 µg					940 ± 8	1634 ± 54

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: 3977311
Experiment: 3977311 HV2 Pre
Assay Conditions:

Study Code: ICCR 3977311
Date Plated: 18.01.2022
Date Counted: 24.01.2022

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		11 ± 4	11 ± 1	29 ± 8	120 ± 12	300 ± 5	371 ± 25
	Untreated		14 ± 3	11 ± 1	30 ± 5	124 ± 8	340 ± 27	344 ± 23
	Thiamethoxam	33 µg	14 ± 3	11 ± 3	27 ± 6	126 ± 14	309 ± 8	344 ± 9
	SL (A23943A)	100 µg	9 ± 1	11 ± 4	32 ± 10	117 ± 7	295 ± 34	400 ± 32
		333 µg	9 ± 2	10 ± 4	26 ± 1	114 ± 2	299 ± 18	364 ± 54
		1000 µg	12 ± 3	17 ± 2	34 ± 7	138 ± 20	310 ± 14	361 ± 12
		2500 µg	15 ± 1	16 ± 5	33 ± 6	127 ± 10	313 ± 9	348 ± 24
		5000 µg	10 ± 3	11 ± 1	39 ± 9	130 ± 8	312 ± 7	319 ± 11
	NaN3	10 µg	1026 ± 74			1763 ± 27		
	4-NOPD	10 µg			579 ± 21			
	4-NOPD	50 µg		120 ± 14				
	MMS	2.0 µL					3055 ± 165	2888 ± 174
With Activation	Deionised water		11 ± 3	15 ± 4	41 ± 4	115 ± 8	311 ± 17	428 ± 25
	Untreated		14 ± 5	16 ± 5	47 ± 10	121 ± 6	340 ± 15	427 ± 19
	Thiamethoxam	33 µg	12 ± 2	17 ± 5	49 ± 12	118 ± 10	320 ± 29	444 ± 24
	SL (A23943A)	100 µg	15 ± 1	17 ± 5	50 ± 10	136 ± 11	321 ± 9	423 ± 23
		333 µg	10 ± 3	15 ± 1	46 ± 9	116 ± 17	340 ± 47	406 ± 22
		1000 µg	15 ± 5	15 ± 6	54 ± 11	151 ± 9	354 ± 48	431 ± 14
		2500 µg	14 ± 4	15 ± 5	55 ± 12	154 ± 9	357 ± 12	392 ± 15
		5000 µg	15 ± 1	16 ± 5	55 ± 7	156 ± 7	319 ± 21	372 ± 5
	2-AA	2.5 µg	261 ± 7	379 ± 29	2164 ± 242	3350 ± 393		
	2-AA	10.0 µg					952 ± 15	1589 ± 72

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/Experiment I: 3977311 VV Plate Incorporation Without Metabolic Activation

Study Name: 3977311
Experiment: 3977311 VV Plate
Assay Conditions:

Study Code: ICCR 3977311
Date Plated: 04.01.2022
Date Counted: 10.01.2022

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Thiamethoxam**	3 µg	*12.7*	*4.0*	*0.9*	9, 17, 12
	SL (A23943A)	10 µg	*11.0*	*1.0*	*0.8*	10, 11, 12
		33 µg	*14.7*	*2.5*	*1.1*	17, 15, 12
		100 µg	*12.7*	*4.0*	*0.9*	12, 17, 9
		333 µg	*12.0*	*2.6*	*0.9*	15, 11, 10
		1000 µg	*8.7*	*2.9*	*0.6*	7, 7, 12
		2500 µg	*9.0*	*1.7*	*0.7*	10, 10, 7
		5000 µg	*14.0*	*2.0*	*1.0*	12 P, 16 P, 14 P
	Deionised water		*13.7*	*1.5*		12, 14, 15
	Untreated		*15.3*	*1.5*		17, 14, 15
TA 1537	**Thiamethoxam**	3 µg	*12.0*	*1.7*	*0.8*	11, 14, 11
	SL (A23943A)	10 µg	*13.3*	*2.9*	*0.9*	15, 15, 10
		33 µg	*10.3*	*2.9*	*0.7*	7, 12, 12
		100 µg	*10.3*	*3.5*	*0.7*	7, 14, 10
		333 µg	*14.0*	*3.6*	*0.9*	10, 17, 15
		1000 µg	*10.3*	*3.5*	*0.7*	7, 10, 14
		2500 µg	*16.3*	*0.6*	*1.0*	17, 16, 16
		5000 µg	*14.7*	*3.2*	*0.9*	16 P, 11 P, 17 P
	Deionised water		*15.7*	*1.5*		14, 17, 16
	Untreated		*12.0*	*3.0*		12, 9, 15
TA 98	**Thiamethoxam**	3 µg	*25.7*	*9.0*	*1.1*	36, 20, 21
	SL (A23943A)	10 µg	*31.0*	*7.8*	*1.3*	40, 27, 26
		33 µg	*28.3*	*8.1*	*1.2*	27, 21, 37
		100 µg	*26.7*	*5.7*	*1.1*	25, 22, 33
		333 µg	*31.7*	*8.1*	*1.3*	26, 41, 28
		1000 µg	*34.3*	*9.1*	*1.4*	44, 26, 33
		2500 µg	*29.0*	*6.6*	*1.2*	22, 35, 30
		5000 µg	*26.0*	*1.0*	*1.1*	27 P, 25 P, 26 P
	Deionised water		*24.3*	*6.1*		23, 19, 31
	Untreated		*29.3*	*7.1*		37, 23, 28

Key to Plate Postfix Codes

P Precipitate

Study Name: 3977311
Experiment: 3977311 VV Plate
Assay Conditions:

Study Code: ICCR 3977311
Date Plated: 04.01.2022
Date Counted: 10.01.2022

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Thiamethoxam**	3 µg	*119.3*	*12.5*	*1.0*	107, 132, 119
	SL (A23943A)	10 µg	*121.3*	*10.6*	*1.0*	123, 131, 110
		33 µg	*112.7*	*2.1*	*1.0*	115, 112, 111
		100 µg	*108.3*	*10.4*	*0.9*	120, 105, 100
		333 µg	*120.3*	*10.4*	*1.0*	132, 112, 117
		1000 µg	*135.3*	*13.5*	*1.2*	135, 122, 149
		2500 µg	*155.3*	*13.6*	*1.3*	141, 157, 168
		5000 µg	*140.7*	*15.5*	*1.2*	136 P, 128 P, 158 P
	Deionised water		*117.7*	*19.9*		122, 96, 135
	Untreated		*125.0*	*3.0*		125, 128, 122
WP2 pKM101	**Thiamethoxam**	3 µg	*305.3*	*6.1*	*1.0*	312, 300, 304
	SL (A23943A)	10 µg	*318.7*	*6.4*	*1.1*	326, 315, 315
		33 µg	*303.7*	*5.5*	*1.0*	310, 300, 301
		100 µg	*313.3*	*16.1*	*1.1*	325, 320, 295
		333 µg	*318.0*	*8.2*	*1.1*	327, 316, 311
		1000 µg	*306.7*	*2.1*	*1.0*	306, 305, 309
		2500 µg	*309.3*	*4.9*	*1.0*	306, 315, 307
		5000 µg	*291.3*	*34.9*	*1.0*	311 P, 251 P, 312 P
	Deionised water		*298.3*	*8.5*		298, 307, 290
	Untreated		*336.7*	*10.7*		349, 330, 331
WP2 uvrA pKM101	**Thiamethoxam**	3 µg	*399.3*	*3.5*	*1.1*	396, 399, 403
	SL (A23943A)	10 µg	*394.0*	*6.0*	*1.0*	394, 400, 388
		33 µg	*394.7*	*4.7*	*1.0*	391, 393, 400
		100 µg	*403.3*	*12.3*	*1.1*	417, 400, 393
		333 µg	*399.7*	*4.0*	*1.1*	399, 404, 396
		1000 µg	*401.3*	*10.3*	*1.1*	390, 410, 404
		2500 µg	*406.3*	*5.5*	*1.1*	400, 409, 410
		5000 µg	*321.7*	*24.0*	*0.8*	336 P, 335 P, 294 P
	Deionised water		*379.3*	*20.1*		385, 396, 357
	Untreated		*394.7*	*4.5*		390, 395, 399
TA 1535	**NaN3**	10 µg	*929.0*	*276.3*	*68.0*	1198, 646, 943
TA 1537	**4-NOPD**	50 µg	*85.3*	*4.2*	*5.4*	90, 82, 84
TA 98	**4-NOPD**	10 µg	*505.0*	*35.8*	*20.8*	521, 530, 464
TA 100	**NaN3**	10 µg	*1509.0*	*261.1*	*12.8*	1245, 1515, 1767
WP2 pKM101	**MMS**	2.0 µL	*3154.0*	*163.8*	*10.6*	3104, 3337, 3021
WP2 uvrA pKM101	**MMS**	2.0 µL	*3532.7*	*277.3*	*9.3*	3393, 3353, 3852

Key to Positive Controls

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

Key to Plate Postfix Codes

P	Precipitate

TABLE 4 Pre-Experiment/Experiment I: 3977311 VV Plate Incorporation With Metabolic Activation

Study Name: 3977311
Experiment: 3977311 VV Plate
Assay Conditions:

Study Code: ICCR 3977311
Date Plated: 04.01.2022
Date Counted: 10.01.2022

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Thiamethoxam**	3 µg	*12.3*	*1.5*	*1.1*	14, 12, 11
	SL (A23943A)	10 µg	*10.3*	*2.9*	*0.9*	12, 7, 12
		33 µg	*11.0*	*1.0*	*0.9*	12, 10, 11
		100 µg	*12.0*	*1.7*	*1.0*	14, 11, 11
		333 µg	*14.3*	*3.8*	*1.2*	16, 10, 17
		1000 µg	*19.0*	*3.6*	*1.6*	20, 22, 15
		2500 µg	*13.3*	*1.2*	*1.1*	12, 14, 14
		5000 µg	*12.0*	*3.6*	*1.0*	11 P, 16 P, 9 P
	Deionised water		*11.7*	*3.1*		9, 15, 11
	Untreated		*10.7*	*0.6*		10, 11, 11
TA 1537	**Thiamethoxam**	3 µg	*16.0*	*3.5*	*1.1*	14, 20, 14
	SL (A23943A)	10 µg	*14.0*	*2.6*	*1.0*	11, 15, 16
		33 µg	*15.0*	*1.0*	*1.0*	14, 15, 16
		100 µg	*15.3*	*5.0*	*1.0*	10, 16, 20
		333 µg	*18.3*	*3.1*	*1.2*	15, 21, 19
		1000 µg	*20.3*	*4.2*	*1.4*	25, 17, 19
		2500 µg	*16.3*	*0.6*	*1.1*	17, 16, 16
		5000 µg	*17.3*	*1.5*	*1.2*	16 P M, 19 P M, 17 P M
	Deionised water		*14.7*	*2.5*		12, 15, 17
	Untreated		*17.3*	*2.5*		17, 15, 20
TA 98	**Thiamethoxam**	3 µg	*50.0*	*3.6*	*1.4*	54, 49, 47
	SL (A23943A)	10 µg	*42.7*	*6.7*	*1.2*	35, 46, 47
		33 µg	*44.0*	*12.2*	*1.2*	36, 58, 38
		100 µg	*40.7*	*9.3*	*1.1*	38, 51, 33
		333 µg	*45.0*	*8.7*	*1.2*	49, 51, 35
		1000 µg	*47.3*	*7.6*	*1.3*	42, 44, 56
		2500 µg	*45.0*	*7.0*	*1.2*	42, 40, 53
		5000 µg	*47.7*	*10.5*	*1.3*	37 P, 48 P, 58 P
	Deionised water		*37.0*	*10.4*		43, 25, 43
	Untreated		*53.0*	*7.5*		61, 52, 46

Key to Plate Postfix Codes

P	Precipitate
M	Manual count

Study Name: 3977311
Experiment: 3977311 VV Plate
Assay Conditions:

Study Code: ICCR 3977311
Date Plated: 04.01.2022
Date Counted: 10.01.2022

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Thiamethoxam**	3 µg	*107.3*	*11.9*	*0.9*	102, 121, 99
	SL (A23943A)	10 µg	*112.3*	*15.6*	*1.0*	127, 96, 114
		33 µg	*118.7*	*6.7*	*1.0*	122, 111, 123
		100 µg	*103.3*	*6.4*	*0.9*	96, 107, 107
		333 µg	*109.7*	*13.9*	*0.9*	106, 98, 125
		1000 µg	*147.7*	*10.5*	*1.3*	137, 148, 158
		2500 µg	*153.0*	*17.3*	*1.3*	172, 138, 149
		5000 µg	*149.7*	*5.9*	*1.3*	152 P, 143 P, 154 P
	Deionised water		*116.7*	*10.4*		105, 125, 120
	Untreated		*110.0*	*14.1*		112, 95, 123
WP2 pKM101	**Thiamethoxam**	3 µg	*328.0*	*22.3*	*1.1*	353, 321, 310
	SL (A23943A)	10 µg	*325.3*	*15.6*	*1.0*	340, 327, 309
		33 µg	*312.3*	*12.7*	*1.0*	305, 305, 327
		100 µg	*314.0*	*14.8*	*1.0*	331, 307, 304
		333 µg	*320.3*	*10.0*	*1.0*	321, 330, 310
		1000 µg	*315.7*	*10.5*	*1.0*	326, 316, 305
		2500 µg	*318.7*	*4.7*	*1.0*	324, 317, 315
		5000 µg	*312.0*	*12.3*	*1.0*	317 P, 298 P, 321 P
	Deionised water		*312.0*	*3.0*		315, 309, 312
	Untreated		*343.0*	*2.6*		341, 342, 346
WP2 uvrA pKM101	**Thiamethoxam**	3 µg	*416.0*	*18.4*	*1.0*	429, 395, 424
	SL (A23943A)	10 µg	*416.0*	*5.0*	*1.0*	421, 416, 411
		33 µg	*423.0*	*8.2*	*1.0*	416, 421, 432
		100 µg	*418.0*	*7.2*	*1.0*	426, 412, 416
		333 µg	*403.7*	*4.0*	*1.0*	406, 399, 406
		1000 µg	*409.0*	*10.5*	*1.0*	420, 408, 399
		2500 µg	*398.3*	*4.6*	*1.0*	401, 393, 401
		5000 µg	*385.3*	*16.2*	*0.9*	368 P, 388 P, 400 P
	Deionised water		*408.3*	*17.0*		389, 415, 421
	Untreated		*469.7*	*6.7*		477, 468, 464
TA 1535	**2-AA**	2.5 µg	*310.7*	*31.4*	*26.6*	300, 346, 286
TA 1537	**2-AA**	2.5 µg	*428.3*	*35.2*	*29.2*	391, 461, 433
TA 98	**2-AA**	2.5 µg	*2982.0*	*326.3*	*80.6*	2607, 3138, 3201
TA 100	**2-AA**	2.5 µg	*3785.3*	*190.6*	*32.4*	3949, 3831, 3576
WP2 pKM101	**2-AA**	10.0 µg	*940.3*	*8.3*	*3.0*	931, 947, 943
WP2 uvrA pKM101	**2-AA**	10.0 µg	*1634.3*	*54.2*	*4.0*	1650, 1574, 1679

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

TABLE 5 Experiment II: 3977311 HV2 Pre Incubation Without Metabolic Activation

Study Name: 3977311 Study Code: ICCR 3977311
Experiment: 3977311 HV2 Pre Date Plated: 18.01.2022
Assay Conditions: Date Counted: 24.01.2022

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Thiamethoxam**	33 µg	*13.7*	*2.5*	*1.3*	14, 16, 11
	SL (A23943A)	100 µg	*9.3*	*0.6*	*0.9*	9, 9, 10
		333 µg	*9.0*	*2.0*	*0.8*	11, 7, 9
		1000 µg	*11.7*	*2.9*	*1.1*	10, 10, 15
		2500 µg	*15.0*	*1.0*	*1.4*	15, 16, 14
		5000 µg	*10.0*	*2.6*	*0.9*	7, 12, 11
	Deionised water		*10.7*	*4.0*		15, 10, 7
	Untreated		*13.7*	*2.5*		11, 14, 16
TA 1537	**Thiamethoxam**	33 µg	*11.3*	*3.2*	*1.0*	10, 9, 15
	SL (A23943A)	100 µg	*10.7*	*3.5*	*0.9*	11, 14, 7
		333 µg	*9.7*	*4.0*	*0.9*	14, 9, 6
		1000 µg	*17.0*	*2.0*	*1.5*	15, 17, 19
		2500 µg	*16.0*	*4.6*	*1.4*	17, 20, 11
		5000 µg	*10.7*	*1.2*	*0.9*	12, 10, 10
	Deionised water		*11.3*	*1.2*		12, 12, 10
	Untreated		*10.7*	*0.6*		10, 11, 11
TA 98	**Thiamethoxam**	33 µg	*26.7*	*5.7*	*0.9*	33, 25, 22
	SL (A23943A)	100 µg	*32.3*	*10.0*	*1.1*	42, 33, 22
		333 µg	*26.3*	*1.2*	*0.9*	27, 25, 27
		1000 µg	*34.0*	*7.2*	*1.2*	32, 28, 42
		2500 µg	*33.3*	*5.5*	*1.1*	27, 37, 36
		5000 µg	*38.7*	*8.6*	*1.3*	48, 37, 31
	Deionised water		*29.3*	*8.1*		38, 28, 22
	Untreated		*30.3*	*5.0*		35, 25, 31
TA 100	**Thiamethoxam**	33 µg	*126.0*	*14.0*	*1.1*	136, 132, 110
	SL (A23943A)	100 µg	*117.0*	*7.0*	*1.0*	122, 120, 109
		333 µg	*114.0*	*2.0*	*1.0*	114, 116, 112
		1000 µg	*138.0*	*19.7*	*1.2*	154, 116, 144
		2500 µg	*127.0*	*9.5*	*1.1*	116, 132, 133
		5000 µg	*129.7*	*7.6*	*1.1*	133, 135, 121
	Deionised water		*120.0*	*12.2*		128, 126, 106
	Untreated		*124.0*	*8.2*		122, 117, 133
WP2 pKM101	**Thiamethoxam**	33 µg	*309.0*	*8.2*	*1.0*	316, 311, 300
	SL (A23943A)	100 µg	*295.0*	*33.8*	*1.0*	326, 300, 259
		333 µg	*299.3*	*17.5*	*1.0*	299, 282, 317
		1000 µg	*310.0*	*14.2*	*1.0*	299, 305, 326
		2500 µg	*313.0*	*9.2*	*1.0*	321, 303, 315
		5000 µg	*312.3*	*7.1*	*1.0*	320, 311, 306
	Deionised water		*299.7*	*4.7*		305, 298, 296
	Untreated		*340.3*	*26.5*		370, 332, 319

Study Name: 3977311 Study Code: ICCR 3977311
Experiment: 3977311 HV2 Pre Date Plated: 18.01.2022
Assay Conditions: Date Counted: 24.01.2022

Without metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2	**Thiamethoxam**	33 µg	*343.7*	*9.0*	*0.9*	353, 343, 335
uvrA	**SL (A23943A)**	100 µg	*399.7*	*32.3*	*1.1*	374, 389, 436
pKM101		333 µg	*364.3*	*53.8*	*1.0*	426, 340, 327
		1000 µg	*361.3*	*12.2*	*1.0*	372, 364, 348
		2500 µg	*347.7*	*23.9*	*0.9*	375, 337, 331
		5000 µg	*318.7*	*11.0*	*0.9*	325, 306, 325
	Deionised water		*371.3*	*24.8*		351, 364, 399
	Untreated		*343.7*	*23.4*		317, 353, 361
TA 1535	**NaN3**	10 µg	*1025.7*	*73.7*	*96.2*	1093, 1037, 947
TA 1537	**4-NOPD**	50 µg	*120.0*	*14.2*	*10.6*	131, 125, 104
TA 98	**4-NOPD**	10 µg	*578.7*	*20.6*	*19.7*	600, 577, 559
TA 100	**NaN3**	10 µg	*1763.3*	*26.7*	*14.7*	1788, 1735, 1767
WP2 pKM101	**MMS**	2.0 µL	*3055.0*	*165.0*	*10.2*	2870, 3108, 3187
WP2 uvrA pKM101	**MMS**	2.0 µL	*2888.0*	*173.6*	*7.8*	2934, 2696, 3034

Key to Positive Controls

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

TABLE 6 Experiment II: 3977311 HV2 Pre Incubation With Metabolic Activation

Study Name: 3977311
Experiment: 3977311 HV2 Pre
Assay Conditions:

Study Code: ICCR 3977311
Date Plated: 18.01.2022
Date Counted: 24.01.2022

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Thiamethoxam**	33 µg	*12.0*	*1.7*	*1.1*	11, 11, 14
	SL (A23943A)	100 µg	*15.0*	*1.0*	*1.3*	15, 14, 16
		333 µg	*9.7*	*3.2*	*0.9*	12, 6, 11
		1000 µg	*14.7*	*4.6*	*1.3*	12, 20, 12
		2500 µg	*14.0*	*3.6*	*1.2*	10, 17, 15
		5000 µg	*14.7*	*0.6*	*1.3*	14, 15, 15
	Deionised water		*11.3*	*3.2*		15, 10, 9
	Untreated		*14.3*	*4.6*		17, 9, 17
TA 1537	**Thiamethoxam**	33 µg	*17.3*	*4.9*	*1.2*	23, 15, 14
	SL (A23943A)	100 µg	*17.0*	*5.3*	*1.1*	11, 21, 19
		333 µg	*15.0*	*1.0*	*1.0*	14, 16, 15
		1000 µg	*15.0*	*5.6*	*1.0*	9, 16, 20
		2500 µg	*15.3*	*4.5*	*1.0*	20, 11, 15
		5000 µg	*16.3*	*4.5*	*1.1*	21, 12, 16
	Deionised water		*15.0*	*3.6*		14, 12, 19
	Untreated		*16.3*	*4.5*		16, 12, 21
TA 98	**Thiamethoxam**	33 µg	*48.7*	*11.9*	*1.2*	57, 35, 54
	SL (A23943A)	100 µg	*50.3*	*9.6*	*1.2*	59, 40, 52
		333 µg	*45.7*	*8.5*	*1.1*	54, 37, 46
		1000 µg	*54.0*	*11.3*	*1.3*	67, 47, 48
		2500 µg	*54.7*	*12.2*	*1.3*	52, 44, 68
		5000 µg	*54.7*	*7.1*	*1.3*	47, 56, 61
	Deionised water		*40.7*	*3.5*		44, 41, 37
	Untreated		*47.0*	*10.1*		56, 36, 49
TA 100	**Thiamethoxam**	33 µg	*118.3*	*10.3*	*1.0*	107, 127, 121
	SL (A23943A)	100 µg	*136.0*	*10.6*	*1.2*	148, 128, 132
		333 µg	*116.0*	*17.3*	*1.0*	107, 136, 105
		1000 µg	*151.0*	*8.9*	*1.3*	144, 161, 148
		2500 µg	*154.0*	*8.9*	*1.3*	161, 157, 144
		5000 µg	*156.3*	*7.0*	*1.4*	163, 149, 157
	Deionised water		*114.7*	*7.8*		117, 106, 121
	Untreated		*121.3*	*6.4*		125, 114, 125
WP2 pKM101	**Thiamethoxam**	33 µg	*320.3*	*29.3*	*1.0*	306, 301, 354
	SL (A23943A)	100 µg	*321.0*	*8.7*	*1.0*	325, 311, 327
		333 µg	*339.7*	*47.2*	*1.1*	295, 335, 389
		1000 µg	*353.7*	*47.9*	*1.1*	406, 312, 343
		2500 µg	*357.0*	*12.5*	*1.1*	343, 367, 361
		5000 µg	*319.0*	*20.8*	*1.0*	331, 295, 331
	Deionised water		*311.3*	*16.8*		326, 315, 293
	Untreated		*340.0*	*14.5*		354, 341, 325

Study Name: 3977311
Experiment: 3977311 HV2 Pre
Assay Conditions:

Study Code: ICCR 3977311
Date Plated: 18.01.2022
Date Counted: 24.01.2022

With metabolic activation

Strain	Compound	Concen-tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2	**Thiamethoxam**	33 µg	*443.7*	*23.7*	*1.0*	469, 422, 440
uvrA	**SL (A23943A)**	100 µg	*423.0*	*22.6*	*1.0*	442, 429, 398
pKM101		333 µg	*405.7*	*21.5*	*0.9*	430, 389, 398
		1000 µg	*431.0*	*14.0*	*1.0*	431, 445, 417
		2500 µg	*392.3*	*14.6*	*0.9*	394, 406, 377
		5000 µg	*371.7*	*4.7*	*0.9*	377, 368, 370
	Deionised water		*428.3*	*24.5*		452, 403, 430
	Untreated		*427.0*	*19.0*		427, 446, 408
TA 1535	**2-AA**	2.5 µg	*261.3*	*6.7*	*23.1*	258, 269, 257
TA 1537	**2-AA**	2.5 µg	*378.7*	*29.3*	*25.2*	352, 374, 410
TA 98	**2-AA**	2.5 µg	*2163.7*	*242.0*	*53.2*	1910, 2392, 2189
TA 100	**2-AA**	2.5 µg	*3349.7*	*392.9*	*29.2*	3576, 3577, 2896
WP2 pKM101	**2-AA**	10.0 µg	*951.7*	*15.0*	*3.1*	935, 956, 964
WP2 uvrA pKM101	**2-AA**	10.0 µg	*1588.7*	*71.6*	*3.7*	1515, 1658, 1593

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 plate incorporation and preincubation methods were used to establish the historical control data since the bacterial strains used in the Ames test do not show differences in their revertant numbers beyond the range of the biological variability in the controls due to the different assay types

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

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

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

APPENDIX 2 Copy of GLP Certificate

HESSEN

Gute Laborpraxis/Good Laboratory Practice

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

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

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

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

ICCR-Roßdorf GmbH
Institute for Competent Contract Research
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

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

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**2** Toxicity studies
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**3** Mutagenicity studies
8 Analytische Prüfungen an biologischen Materialien	**8** Analytical and clinical chemistry testing

22.11.2018, 21.02.2019, 12. bis 14.03.2019
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

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

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

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

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

Im Auftrag

Brandt

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

HESSISCHES MINISTERIUM FÜR UMWELT, KLIMASCHUTZ, LANDWIRTSCHAFT UND VERBRAUCHERSCHUTZ

Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80, D 65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority)

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

APPENDIX 3 Certificate of S9

ICCR ROßDORF

CERTIFICATE

ICCR-Roßdorf S9 Preparation Lot No. 080721D

Date of preparation: July 08, 2021
Release date: July 26, 2021

Protein assay: 29.6 mg protein / ml S9

Sterility: 7.6 colonies / ml S9 on glucose-minimal-agar

Salmonella typhimurium assay (AMES-test)

Treatment	µl S9 / plate	number of revertants in TA 98
negative	0	33
control	100	54
10 µg/plate	0	40
2-Aminoanthracene	100	2031
10 µg/plate	0	23
Benzo(a)pyrene	100	88

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

S. Ebert Sabine Ebert — 27. JULI 2021
Quality Assurance Auditor — Date
ICCR-Roßdorf GmbH

27. JULI 2021
Dr. Steffen Naumann — Date
Study Director
ICCR-Roßdorf GmbH

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

SOP Origin TS-SOP S9_23

Best available copy

APPENDIX 4 Certificate of Analysis

ALS Laboratórios LS Ltda.
Rua Fábia, 59 – CEP: 05051-030
São Paulo, SP - Brazil

SYNGENTA PROTEÇÃO DE CULTIVOS Ltda.
Rua Doutor Rubens Gomes Bueno nº 691,
11º andar, Torre Sigma
CEP 04730-000 – Bairro Várzea de Baixo
São Paulo-SP – Brazil

Certificate of Analysis

A23943A
Thiamethoxam SL (075)
NSI001-085-017

Batch Identification	NSI001-085-017
Product Code	A23943A
Other Product Code(s)	A23943A; EXF23867A; CGA293343 SL (075); Thiamethoxam SL (075)
EUP number:	739/2021 Expiry date: 26/04/2024
Received on:	28 September 2021
Source	Syngenta Proteção de Cultivos Ltda. Rodovia Professor Zeferino Vaz, SP 332, s/nº, km 127,5 – Bairro Santa Terezinha, CEP 13148-915 – Paulínia-SP – Brasil
Chemical Analysis (Active Ingredients Content)	
– **Content of Thiamethoxam ***	**6.63 % w/w corresponding to 75.42 g/L**

The Active Ingredient content is within the FAO limits.
Methodology used for Characterization: HPLC (SF-1151/1)

Physical Analysis	
– **Appearance**	Homogeneous and translucent
– **Color**	6/12 – 5YR (Dark Orange)
– **Physical state**	Liquid
– **Density ***	1.1378 g/cm^3
Stability:	
– **Storage Temperature**	**<30°C**
– **Recertification Date**	**10 September 2023**

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

This Certificate of Analysis summarizes data which originates either from a single study or from several individual studies. Tests marked with an asterisk (*) have been conducted in compliance with GLP. All original raw data, including any storage medium for electronically recorded data, documentation, the signed study plan, the protocol amendments, the final report and a sample of the test item will be retained in the GLP Archives at ALS Laboratórios LS Ltda.

Study number of batch characterization: 26785/2021CF and 26787/2021CC

Authorization: 10 November 2021

Victor F. G. da Silva
Victor Ferreira Gomes da Silva
ALS Laboratórios LS Ltda.

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