

Isocycloseram/Emamectin Benzoate

**Isocycloseram/Emamectin Benzoate SC (A23220A) -
Salmonella Typhimurium and *Escherichia Coli* Reverse
Mutation Assay**

Final Report

TEST GUIDELINE(S): OECD 471 (1997)

AUTHOR(S): Dr. Steffi Chang

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

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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


.....
Date: 20 July 2020

Performing Laboratory:
ICCR-Roßdorf GmbH
In den Leppsteinswiesen 19
64380 Rossdorf, Germany

To be completed for USA EPA submission only:
Representative of Submitter/Sponsor:

_____	_____
	Date
Submitter/Sponsor: Syngenta Crop Protection, LLC	
410 Swing Road	
Post Office Box 18300	
Greensboro, NC 27419-8300 USA	

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

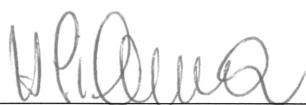
ICCR Study Number: 2106900
Test substance: Isocycloseram/Emamectin Benzoate SC (A23220A)
Study director: Dr. Steffi Chang
Study Title: Isocycloseram/Emamectin Benzoate SC (A23220A) -
Salmonella Typhimurium and
Escherichia Coli Reverse Mutation Assay

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

Type of Inspection	Date(s) of Inspection	Date Reporting to Study Director, Test Facility Management
Study Plan Verification	23 April 2020	23 April 2020
Process – based Assessment of Response	13 May 2020	13 May 2020
Report Audit	25 June 2020	25 June 2020

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

Quality Assurance Auditor
ICCR-Roßdorf GmbH

20 July 2020

Date

PROJECT STAFF SIGNATURE

Study Director

Dr. Steffi Chang

A handwritten signature in black ink, appearing to be 'S. Chang', written over a dotted line.

Date: 20 July 2020

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
Sophie van der Kamp	Syngenta Study Manager

Study Dates

Study initiation date:	24 April 2020
Experimental start date:	30 April 2020
Experimental completion date:	04 June 2020

Deviations from the Guidelines

None

Retention of Samples

None

Performing Laboratory Test Substance Reference Number

S 2092211

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

The following deviations from GSP471.Ames.Syngenta.10 occurred:

3.9 Major Computerized Systems

The manufacturer of the major computerized system Petri Viewer Sorcerer Colony Counter has changed it`s company name to Instem.

Appendix 1: Historical Control Data

These data represent the laboratory`s historical control data from November 2016 until August 2018 representing approx. 600 experiments (WP2 pKM101, WP2 uvrA pKM101 the historical data are based on approx. 60 experiments).

These deviations are considered to have not affected the integrity or validity of the study.

Distribution of the report

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

TABLE OF CONTENTS

STATEMENT OF DATA CONFIDENTIALITY CLAIMS	2
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	3
FLAGGING STATEMENT	4
QUALITY ASSURANCE STATEMENT	5
PROJECT STAFF SIGNATURE	6
GENERAL INFORMATION	7
TABLE OF CONTENTS	9
1.0 EXECUTIVE SUMMARY	11
1.1 Study Design	11
1.2 Results	11
1.3 Conclusion.....	11
2.0 INTRODUCTION	12
2.1 Purpose	12
2.2 Test Guideline(s).....	12
3.0 MATERIALS AND METHODS	13
3.1 Test Substance.....	13
3.2 Controls	14
3.2.1 Negative controls	14
3.2.2 Positive control substances	14
3.3 Experimental Design.....	15
3.3.1 Characterisation of the <i>Salmonella typhimurium</i> and <i>E. coli</i> strains	15
3.3.2 Storage.....	16
3.3.3 Precultures.....	16
3.3.4 Selective agar	16
3.3.5 Overlay agar	16
3.4 Mammalian Microsomal Fraction S9 Mix.....	16
3.4.1 S9 (Preparation by ICCR-Roßdorf GmbH).....	17
3.4.2 S9 mix	17
3.5 Pre-Experiment for Cytotoxicity	17
3.6 Concentration Selection	18
3.7 Experimental Performance.....	18
3.8 Data Evaluation	18
3.8.1 Data recording	18

3.8.2	Acceptability of the assay	19
3.8.3	Evaluation of results.....	19
3.8.4	Biometry.....	19
3.9	Major Computerized System.....	19
4.0	RESULTS AND DISCUSSION	20
5.0	CONCLUSIONS	21
6.0	REFERENCES	22
TABLES SECTION		23
TABLE 1	Summary of Results Pre-Experiment/Experiment I.....	24
TABLE 2	Summary of Results Experiment II.....	25
TABLE 3	Pre-Experiment and Experiment I: 2106900 VV Plate Incorporation Without Metabolic Activation.....	26
TABLE 4	Pre-Experiment and Experiment I: 2106900 VV Plate Incorporation With Metabolic Activation.....	28
TABLE 5	Experiment II: 2106900 HV2 Pre Incubation Without Metabolic Activation.....	30
TABLE 6	Experiment II: 2106900 HV2 Pre Incubation With Metabolic Activation.....	32
APPENDICES SECTION		34
APPENDIX 1	Historical Control Data	35
APPENDIX 2	Copy of GLP Certificate	36
APPENDIX 3	Certificate of S9	37
APPENDIX 4	Certificate of Analysis.....	38

1.0 EXECUTIVE SUMMARY

1.1 Study Design

This study was performed to investigate the potential of Isocycloseram/Emamectin Benzoate SC (A23220A) 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.

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 Isocycloseram/Emamectin Benzoate SC (A23220A) 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 confirming the correct performance of the assay and the activity of the S9-mix.

1.3 Conclusion

In conclusion, it can be stated that during the described mutagenicity tests and under the experimental conditions reported, Isocycloseram/Emamectin Benzoate SC (A23220A) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, Isocycloseram/Emamectin Benzoate SC (A23220A) 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 $\text{his}^- \rightarrow \text{his}^+$ and $\text{trp}^- \rightarrow \text{trp}^+$ 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”, adopted July 21, 1997

3.0 MATERIALS AND METHODS

3.1 Test Substance

Information as provided by the Sponsor.

Identification:	Isocycloseram/Emamectin Benzoate SC (A23220A)
Batch:	TSC002-041-001
Content of isocycloseram:	17.5% w/w corresponding to 201 g/L
Content of emamectin benzoate:	4.18% w/w corresponding to 48.1 g/L
Appearance:	Brown liquid
Recertification Date:	31 January 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 Isocycloseram/Emamectin Benzoate SC (A23220A).

On the day of the experiment (immediately before use), the test substance was suspended in deionised water. The solvent was chosen as the best 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, (NaN₃)
Supplier: SERVA, 69042 Heidelberg, Germany
Batch No.: 150564
Purity: ≥ 99%
Dissolved in: Deionised water
Concentration: 10 µg/plate

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

Strains: WP2 *uvrA* (pKM101), WP2 (pKM101)
Name: Methyl methane sulfonate, (MMS)
Supplier: Sigma-Aldrich, 82024 Taufkirchen, Germany
Batch No.: MKCG 1346
Purity: ≥ 99%
Dissolved in: Deionised water
Concentration: 2.0 µL/plate

With metabolic activation

Strains: TA1535, TA1537, TA98, TA100, WP2 *uvrA* (pKM101), WP2 (pKM 101)
Name: 2-aminoanthracene, (2-AA)
Supplier: Sigma-Aldrich, 82024 Taufkirchen, Germany
Batch No.: STBG 0630V
Purity: ≥ 96%
Dissolved in: DMSO (purity > 99 %, Fisher Leics LE11 5RG, United Kingdom)
Concentration: 2.5 µg/plate (TA1535, TA1537, TA98, TA100),
10 µg/plate (WP2 *uvrA* (pKM101), WP2 (pKM101))

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range is sufficient evidence of biological activity.

3.3 Experimental Design

3.3.1 Characterisation of the *Salmonella typhimurium* and *E. coli* strains

The histidine dependent strains are derived from *S. typhimurium* strain LT2 through mutations in the histidine locus. Additionally, due to the "deep rough" (*rfa*⁻) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The last alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named *uvrB*⁻. In the strains TA98 and TA100 the R-factor plasmid pKM101 carries the ampicillin resistance marker (3).

Strain WP2 (4) and its derivatives all carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (*Trp*⁺) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another. Additionally, the *uvrA* derivative is deficient in the DNA repair process (excisable repair damage). Such a repair-deficient strain may be more readily mutated by agents. The *E. coli* strains WP2 *uvrA* (pKM101) and WP2 (pKM101) are constructed by introduction of the R-factor plasmid pKM101.

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

Strains	Genotype	Type of mutations indicated
<i>Salmonella typhimurium</i>		
TA1537	<i>his</i> C 3076; <i>rfa</i> ⁻ ; <i>uvrB</i> ⁻	frame shift mutations
TA98	<i>his</i> D 3052; <i>rfa</i> ⁻ ; <i>uvrB</i> ⁻ ; R-factor	" "
TA1535	<i>his</i> G 46; <i>rfa</i> ⁻ ; <i>uvrB</i> ⁻	base-pair substitutions
TA100	<i>his</i> G 46; <i>rfa</i> ⁻ ; <i>uvrB</i> ⁻ ; R-factor	" "
<i>Escherichia coli</i>		
WP2 <i>uvrA</i> (pKM101)	<i>trp</i> E 56 <i>uvrA</i> ⁻ ; R-factor	base-pair substitutions and others
WP2 (pKM101)	<i>trp</i> 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:

7.0 g Agar Agar*

6.0 g NaCl*

10.5 mg L-Histidine×HCl×H₂O*

12.2 mg Biotin*

for *Escherichia coli* strains:

7.0 g Agar Agar*

6.0 g NaCl*

10.2 mg Tryptophan*

* (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/ β -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 β -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 29.7 mg/mL (lot no. 281119B) 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:

8mM MgCl_2
33mM KCl
5mM Glucose-6-phosphate
4mM 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. 8×10^8 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 $37\text{ C} \pm 1.5^\circ\text{ C}$ 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 $37\text{ C} \pm 1.5^\circ\text{ C}$ 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.

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, Isocycloseram/Emamectin Benzoate SC (A23220A), 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.

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

The test item precipitated in the overlay agar in the test tubes from 1000 to 5000 µg/plate. In Experiment I precipitation of the test item occurred in the overlay agar on the incubated agar plates at 5000 µg/plate. The undissolved particles had no influence on the data recording. In Experiment II no precipitation of the test substance occurred in the overlay agar on the incubated agar plates.

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.

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 Isocycloseram/Emamectin Benzoate SC (A23220A) 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 confirming the correct performance of the assay and the activity of the S9-mix.

5.0 CONCLUSIONS

In conclusion, it can be stated that during the described mutagenicity tests and under the experimental conditions reported, Isocycloseram/Emamectin Benzoate SC (A23220A) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, Isocycloseram/Emamectin Benzoate SC (A23220A) 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: 2106900
Experiment: 2106900 VV Plate
Assay Conditions:

Study Code: ICCR 2106900
Date Plated: 30.04.2020
Date Counted: 07.05.2020

Metabolic Activation	Test Group	Concentration (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	Deionised water		11 ± 1	14 ± 1	29 ± 8	92 ± 11	265 ± 22	334 ± 8
	Untreated		12 ± 4	15 ± 5	26 ± 7	93 ± 9	278 ± 13	356 ± 25
	Isocycloseram/	3 µg	8 ± 2	15 ± 1	30 ± 9	91 ± 6	272 ± 32	347 ± 17
	Emamectin	10 µg	9 ± 2	12 ± 3	30 ± 3	88 ± 11	257 ± 20	353 ± 6
	Benzoate SC	33 µg	10 ± 2	12 ± 4	33 ± 11	109 ± 12	277 ± 16	362 ± 20
	(A23220A)	100 µg	11 ± 3	11 ± 1	28 ± 3	92 ± 13	261 ± 9	362 ± 5
		333 µg	7 ± 2	10 ± 1	31 ± 12	112 ± 16	281 ± 17	347 ± 5
		1000 µg	9 ± 3	10 ± 1	21 ± 8	118 ± 1	307 ± 9	356 ± 10
		2500 µg	10 ± 3	10 ± 4	21 ± 4	97 ± 4	261 ± 2	347 ± 18
		5000 µg	8 ± 2 ^P	9 ± 2 ^P	21 ± 4 ^P	98 ± 6 ^P	231 ± 33 ^P	302 ± 9 ^P
	NaN3	10 µg	1088 ± 7			1654 ± 33		
	4-NOPD	10 µg			358 ± 35			
	4-NOPD	50 µg		78 ± 5				
	MMS	2.0 µL					3510 ± 26	3389 ± 61
With Activation	Deionised water		13 ± 6	16 ± 4	34 ± 6	89 ± 10	287 ± 27	393 ± 11
	Untreated		11 ± 1	13 ± 4	42 ± 3	91 ± 10	298 ± 22	373 ± 13
	Isocycloseram/	3 µg	12 ± 2	14 ± 3	46 ± 5	82 ± 10	299 ± 5	381 ± 17
	Emamectin	10 µg	13 ± 5	16 ± 5	37 ± 12	83 ± 11	312 ± 20	373 ± 14
	Benzoate SC	33 µg	9 ± 2	13 ± 3	42 ± 1	88 ± 9	271 ± 12	383 ± 14
	(A23220A)	100 µg	14 ± 3	13 ± 2	43 ± 7	96 ± 15	265 ± 24	403 ± 19
		333 µg	12 ± 3	16 ± 5	41 ± 6	101 ± 6	277 ± 19	391 ± 36
		1000 µg	13 ± 1	15 ± 4	38 ± 6	114 ± 2	264 ± 31	403 ± 30
		2500 µg	11 ± 1	13 ± 2	34 ± 4	133 ± 7	260 ± 16	345 ± 7
		5000 µg	14 ± 2 ^P	12 ± 3 ^P	37 ± 4 ^P	92 ± 8 ^P	292 ± 5 ^P	349 ± 31 ^P
	2-AA	2.5 µg	270 ± 20	377 ± 21	2983 ± 308	2795 ± 96		
	2-AA	10.0 µg					1087 ± 15	1991 ± 109
Key to Positive Controls			Key to Plate Postfix Codes					
NaN3	sodium azide			P	Precipitate			
2-AA	2-aminoanthracene							
4-NOPD	4-nitro-o-phenylene-diamine							
MMS	methyl methane sulfonate							

TABLE 2 Summary of Results Experiment II

Study Name: 2106900
Experiment: 2106900 HV2 Pre
Assay Conditions:

Study Code: ICCR 2106900
Date Plated: 28.05.2020
Date Counted: 04.06.2020

Metabolic Activation	Test Group	Concentration (per plate)	Revertant Colony Counts (Mean $\pm$ SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	Deionised water		9 $\pm$ 1	10 $\pm$ 3	25 $\pm$ 7	100 $\pm$ 6	262 $\pm$ 6	325 $\pm$ 22
	Untreated		9 $\pm$ 2	13 $\pm$ 3	32 $\pm$ 2	96 $\pm$ 12	252 $\pm$ 15	301 $\pm$ 3
	Isocycloseram/	33 μ g	9 $\pm$ 0	13 $\pm$ 1	34 $\pm$ 7	120 $\pm$ 13	266 $\pm$ 11	335 $\pm$ 28
	Emamectin	100 μ g	11 $\pm$ 1	14 $\pm$ 3	27 $\pm$ 12	102 $\pm$ 18	273 $\pm$ 6	364 $\pm$ 30
	Benzoate SC	333 μ g	13 $\pm$ 4	13 $\pm$ 2	33 $\pm$ 5	120 $\pm$ 9	249 $\pm$ 13	330 $\pm$ 8
	(A23220A)	1000 μ g	12 $\pm$ 2	9 $\pm$ 2	27 $\pm$ 6	101 $\pm$ 6	272 $\pm$ 8	316 $\pm$ 14
		2500 μ g	9 $\pm$ 2	13 $\pm$ 2	18 $\pm$ 4	106 $\pm$ 18	262 $\pm$ 19	289 $\pm$ 16
		5000 μ g	10 $\pm$ 1	8 $\pm$ 1	20 $\pm$ 5	100 $\pm$ 10	266 $\pm$ 8	297 $\pm$ 5
	NaN3	10 μ g	1102 $\pm$ 90			1858 $\pm$ 170		
	4-NOPD	10 μ g			939 $\pm$ 81			
	4-NOPD	50 μ g		92 $\pm$ 9				
	MMS	2.0 μ L					2947 $\pm$ 610	3187 $\pm$ 36
With Activation	Deionised water		14 $\pm$ 3	16 $\pm$ 4	36 $\pm$ 1	101 $\pm$ 23	298 $\pm$ 15	331 $\pm$ 32
	Untreated		17 $\pm$ 4	15 $\pm$ 4	37 $\pm$ 1	99 $\pm$ 11	304 $\pm$ 10	373 $\pm$ 18
	Isocycloseram/	33 μ g	14 $\pm$ 2	17 $\pm$ 3	43 $\pm$ 3	99 $\pm$ 25	279 $\pm$ 38	375 $\pm$ 16
	Emamectin	100 μ g	13 $\pm$ 3	20 $\pm$ 1	46 $\pm$ 4	118 $\pm$ 21	270 $\pm$ 17	364 $\pm$ 10
	Benzoate SC	333 μ g	14 $\pm$ 2	16 $\pm$ 4	42 $\pm$ 9	130 $\pm$ 23	258 $\pm$ 19	386 $\pm$ 29
	(A23220A)	1000 μ g	13 $\pm$ 2	13 $\pm$ 2	45 $\pm$ 12	133 $\pm$ 6	313 $\pm$ 12	390 $\pm$ 17
	Isocycloseram/	2500 μ g	15 $\pm$ 3	16 $\pm$ 5	28 $\pm$ 9	132 $\pm$ 10	302 $\pm$ 37	380 $\pm$ 2
		5000 μ g	13 $\pm$ 1	15 $\pm$ 4	36 $\pm$ 5	98 $\pm$ 7	314 $\pm$ 40	368 $\pm$ 23
	2-AA	2.5 μ g	289 $\pm$ 6	349 $\pm$ 36	3518 $\pm$ 298	2318 $\pm$ 231		
	2-AA	10.0 μ g					1037 $\pm$ 21	1739 $\pm$ 73

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: 2106900 VV Plate
Incorporation Without Metabolic Activation**

Study Name: 2106900
Experiment: 2106900 VV Plate
Assay Conditions:

Study Code: ICCR 2106900
Date Plated: 30.04.2020
Date Counted: 07.05.2020

Without metabolic activation						
Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Isocycloseram/	3 µg	8.3	2.3	0.7	7, 11, 7
	Emamectin	10 µg	9.0	2.0	0.8	9, 11, 7
	Benzoate SC	33 µg	10.0	1.7	0.9	9, 9, 12
	(A23220A)	100 µg	11.0	3.5	1.0	9, 15, 9
		333 µg	7.3	1.5	0.6	9, 7, 6
		1000 µg	9.3	2.5	0.8	9, 7, 12
		2500 µg	10.3	2.9	0.9	12, 7, 12
		5000 µg	7.7	2.1	0.7	10 P, 7 P, 6 P
	Deionised water		11.3	0.6		12, 11, 11
	Untreated Control		11.7	3.8		10, 16, 9
TA 1537	Isocycloseram/	3 µg	15.0	1.0	1.0	14, 15, 16
	Emamectin	10 µg	12.3	2.5	0.9	12, 10, 15
	Benzoate SC	33 µg	12.0	4.4	0.8	10, 9, 17
	(A23220A)	100 µg	11.0	1.0	0.8	12, 10, 11
		333 µg	10.3	0.6	0.7	11, 10, 10
		1000 µg	10.0	1.0	0.7	10, 11, 9
		2500 µg	10.3	3.5	0.7	7, 10, 14
		5000 µg	8.7	2.3	0.6	6 P, 10 P, 10 P
	Deionised water		14.3	0.6		14, 15, 14
	Untreated		14.7	4.5		10, 15, 19
TA 98	Isocycloseram/	3 µg	30.3	8.7	1.1	23, 28, 40
	Emamectin	10 µg	30.3	3.1	1.1	33, 27, 31
	Benzoate SC	33 µg	33.0	10.6	1.2	41, 37, 21
	(A23220A)	100 µg	28.0	3.0	1.0	31, 25, 28
		333 µg	30.7	11.6	1.1	25, 44, 23
		1000 µg	20.7	8.1	0.7	30, 15, 17
		2500 µg	20.7	4.0	0.7	20, 25, 17
		5000 µg	21.3	4.0	0.7	25 P, 17 P, 22 P
	Deionised water		28.7	8.0		21, 28, 37
	Untreated		26.0	6.6		25, 20, 33
Key to Plate Postfix Codes						
P						Precipitate

Study Name: 2106900
 Experiment: 2106900 VV Plate
 Assay Conditions:

Study Code: ICCR 2106900
 Date Plated: 30.04.2020
 Date Counted: 07.05.2020

Without metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	Isocycloseram/	3 µg	91.3	5.5	1.0	94, 85, 95
	Emamectin	10 µg	88.0	11.1	1.0	86, 78, 100
	Benzoate SC	33 µg	109.0	12.1	1.2	120, 111, 96
	(A23220A)	100 µg	92.0	13.1	1.0	101, 98, 77
		333 µg	112.3	16.2	1.2	102, 104, 131
		1000 µg	118.3	1.2	1.3	119, 119, 117
		2500 µg	97.0	4.4	1.1	94, 95, 102
		5000 µg	97.7	5.5	1.1	104 P, 95 P, 94 P
	Deionised water		92.3	10.7		90, 104, 83
	Untreated		93.3	9.3		101, 83, 96
WP2 pKM101	Isocycloseram/	3 µg	272.0	32.4	1.0	237, 301, 278
	Emamectin	10 µg	257.3	20.1	1.0	274, 235, 263
	Benzoate SC	33 µg	276.7	15.5	1.0	283, 288, 259
	(A23220A)	100 µg	260.7	9.1	1.0	262, 269, 251
		333 µg	280.7	17.0	1.1	261, 290, 291
		1000 µg	306.7	9.1	1.2	317, 300, 303
		2500 µg	261.0	2.0	1.0	261, 259, 263
		5000 µg	231.3	32.9	0.9	259 P, 240 P, 195 P
	Deionised water		264.7	22.2		241, 268, 285
	Untreated		278.3	12.7		270, 293, 272
WP2 uvrA pKM101	Isocycloseram/	3 µg	347.3	17.0	1.0	367, 338, 337
	Emamectin	10 µg	352.7	5.7	1.1	351, 359, 348
	Benzoate SC	33 µg	362.3	20.3	1.1	346, 356, 385
	(A23220A)	100 µg	361.7	5.0	1.1	367, 357, 361
		333 µg	347.0	5.0	1.0	352, 342, 347
		1000 µg	356.0	10.0	1.1	346, 356, 366
		2500 µg	347.3	17.6	1.0	366, 331, 345
		5000 µg	302.3	9.0	0.9	293 P, 303 P, 311 P
	Deionised water		333.7	8.3		327, 331, 343
	Untreated		356.3	25.1		330, 359, 380
TA 1535	NaN3	10 µg	1088.3	6.7	96.0	1081, 1094, 1090
TA 1537	4-NOPD	50 µg	77.7	5.0	5.4	83, 77, 73
TA 98	4-NOPD	10 µg	358.3	34.6	12.5	362, 391, 322
TA 100	NaN3	10 µg	1654.3	33.3	17.9	1642, 1629, 1692
WP2 pKM101	MMS	2.0 µL	3510.3	26.4	13.3	3505, 3487, 3539
WP2 uvrA pKM101	MMS	2.0 µL	3389.0	60.6	10.2	3454, 3379, 3334
Key to Positive Controls						Key to Plate Postfix Codes
NaN3	sodium azide					P Precipitate
4-NOPD	4-nitro-o-phenylene-diamine					
MMS	methyl methane sulfonate					

**TABLE 4 Pre-Experiment and Experiment I: 2106900 VV Plate
Incorporation With Metabolic Activation**

Study Name: 2106900
Experiment: 2106900 VV Plate
Assay Conditions:

Study Code: ICCR 2106900
Date Plated: 30.04.2020
Date Counted: 07.05.2020

With metabolic activation

Strain	Compound	Concen- tration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Isocycloseram/	3 µg	12.3	1.5	0.9	11, 14, 12
	Emamectin	10 µg	13.3	4.9	1.0	10, 11, 19
	Benzoate SC	33 µg	9.3	2.1	0.7	11, 7, 10
	(A23220A)	100 µg	13.7	3.2	1.0	15, 10, 16
		333 µg	12.3	2.9	0.9	14, 9, 14
		1000 µg	12.7	1.2	0.9	12, 14, 12
		2500 µg	11.0	1.0	0.8	12, 10, 11
		5000 µg	13.7	1.5	1.0	15 P, 14 P, 12 P
	Deionised water		13.3	5.8		20, 10, 10
	Untreated		11.3	1.2		10, 12, 12
TA 1537	Isocycloseram/	3 µg	13.7	3.2	0.8	10, 15, 16
	Emamectin	10 µg	16.3	4.6	1.0	19, 19, 11
	Benzoate SC	33 µg	13.0	3.5	0.8	11, 17, 11
	(A23220A)	100 µg	12.7	2.1	0.8	11, 15, 12
		333 µg	16.0	4.6	1.0	17, 11, 20
		1000 µg	15.0	4.0	0.9	19, 11, 15
		2500 µg	13.3	2.3	0.8	16, 12, 12
		5000 µg	12.3	2.9	0.8	14 P, 9 P, 14 P
	Deionised water		16.3	4.0		21, 14, 14
	Untreated		12.7	3.8		11, 10, 17
TA 98	Isocycloseram/	3 µg	46.0	5.0	1.4	46, 51, 41
	Emamectin	10 µg	37.0	12.5	1.1	27, 33, 51
	Benzoate SC	33 µg	42.0	1.0	1.2	42, 43, 41
	(A23220A)	100 µg	42.7	7.4	1.3	40, 51, 37
		333 µg	40.7	5.5	1.2	38, 47, 37
		1000 µg	38.0	6.2	1.1	31, 40, 43
		2500 µg	33.7	4.0	1.0	33, 30, 38
		5000 µg	37.3	4.0	1.1	35 P, 42 P, 35 P
	Deionised water		33.7	5.9		36, 27, 38
	Untreated		42.3	3.2		46, 41, 40
Key to Plate Postfix Codes						
P						Precipitate

Study Name: 2106900
 Experiment: 2106900 VV Plate
 Assay Conditions:

Study Code: ICCR 2106900
 Date Plated: 30.04.2020
 Date Counted: 07.05.2020

With metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	Isocycloseram/	3 µg	82.0	9.5	0.9	72, 91, 83
	Emamectin	10 µg	83.3	11.1	0.9	82, 73, 95
	Benzoate SC	33 µg	88.3	9.0	1.0	93, 94, 78
	(A23220A)	100 µg	95.7	14.6	1.1	102, 79, 106
		333 µg	101.3	5.5	1.1	104, 105, 95
		1000 µg	113.7	1.5	1.3	114, 115, 112
		2500 µg	133.3	6.8	1.5	128, 131, 141
		5000 µg	91.7	8.3	1.0	89 P, 101 P, 85 P
	Deionised water		89.0	10.0		79, 99, 89
	Untreated		90.7	10.2		95, 98, 79
WP2 pKM101	Isocycloseram/	3 µg	299.0	5.3	1.0	301, 303, 293
	Emamectin	10 µg	312.0	20.1	1.1	335, 298, 303
	Benzoate SC	33 µg	270.7	12.1	0.9	280, 257, 275
	(A23220A)	100 µg	264.7	24.2	0.9	237, 282, 275
		333 µg	277.3	18.6	1.0	256, 286, 290
		1000 µg	264.0	31.2	0.9	268, 231, 293
		2500 µg	260.3	16.2	0.9	275, 243, 263
		5000 µg	291.7	4.9	1.0	295 P, 286 P, 294 P
	Deionised water		287.0	27.2		283, 316, 262
	Untreated		298.0	22.3		316, 305, 273
WP2 uvrA pKM101	Isocycloseram/	3 µg	381.3	16.9	1.0	377, 367, 400
	Emamectin	10 µg	372.7	13.7	0.9	385, 375, 358
	Benzoate SC	33 µg	382.7	13.5	1.0	369, 383, 396
	(A23220A)	100 µg	403.0	19.1	1.0	405, 421, 383
		333 µg	391.0	36.0	1.0	426, 393, 354
		1000 µg	403.3	29.9	1.0	393, 380, 437
		2500 µg	345.0	7.0	0.9	353, 340, 342
		5000 µg	348.7	30.7	0.9	375 P, 315 P, 356 P
	Deionised water		392.7	11.0		399, 399, 380
	Untreated		373.0	13.2		368, 388, 363
TA 1535	2-AA	2.5 µg	270.0	19.5	20.2	290, 269, 251
TA 1537	2-AA	2.5 µg	376.7	20.5	23.1	388, 353, 389
TA 98	2-AA	2.5 µg	2983.0	308.4	88.6	2934, 2702, 3313
TA 100	2-AA	2.5 µg	2794.7	96.4	31.4	2906, 2739, 2739
WP2 pKM101	2-AA	10.0 µg	1087.0	14.7	3.8	1090, 1100, 1071
WP2 uvrA pKM101	2-AA	10.0 µg	1991.3	108.8	5.1	1928, 1929, 2117

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate

TABLE 5 Experiment II: 2106900 HV2 Pre Incubation Without Metabolic Activation

Study Name: 2106900
Experiment: 2106900 HV2 Pre
Assay Conditions:

Study Code: ICCR 2106900
Date Plated: 28.05.2020
Date Counted: 04.06.2020

Without metabolic activation						
Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Isocycloseram/	33 µg	9.0	0.0	1.0	9, 9, 9
	Emamectin	100 µg	10.7	1.2	1.1	12, 10, 10
	Benzoate SC	333 µg	13.0	3.6	1.4	12, 10, 17
	(A23220A)	1000 µg	11.7	2.1	1.2	10, 14, 11
		2500 µg	8.7	1.5	0.9	9, 7, 10
		5000 µg	10.3	1.2	1.1	11, 11, 9
	Deionised water		9.3	0.6		10, 9, 9
	Untreated		9.0	2.0		9, 7, 11
TA 1537	Isocycloseram/	33 µg	12.7	1.2	1.2	14, 12, 12
	Emamectin	100 µg	13.7	3.2	1.3	10, 16, 15
	Benzoate SC	333 µg	12.7	2.1	1.2	11, 12, 15
	(A23220A)	1000 µg	8.7	1.5	0.8	9, 7, 10
		2500 µg	12.7	2.1	1.2	12, 15, 11
		5000 µg	7.7	1.2	0.7	7, 7, 9
	Deionised water		10.3	2.9		7, 12, 12
	Untreated		13.3	3.1		10, 16, 14
TA 98	Isocycloseram/	33 µg	34.3	7.1	1.4	33, 28, 42
	Emamectin	100 µg	26.7	11.5	1.1	20, 40, 20
	Benzoate SC	333 µg	32.7	5.0	1.3	28, 38, 32
	(A23220A)	1000 µg	27.3	5.5	1.1	33, 27, 22
		2500 µg	18.0	4.4	0.7	15, 23, 16
		5000 µg	20.0	5.2	0.8	23, 14, 23
	Deionised water		25.0	7.0		33, 20, 22
	Untreated		32.3	2.3		31, 35, 31
TA 100	Isocycloseram/	33 µg	119.7	12.7	1.2	127, 105, 127
	Emamectin	100 µg	101.7	17.8	1.0	98, 86, 121
	Benzoate SC	333 µg	119.7	9.1	1.2	110, 121, 128
	(A23220A)	1000 µg	101.3	5.5	1.0	101, 107, 96
		2500 µg	106.0	18.3	1.1	86, 110, 122
		5000 µg	100.3	10.0	1.0	101, 110, 90
	Deionised water		100.3	5.9		98, 96, 107
	Untreated		96.3	11.7		101, 105, 83

Study Name: 2106900
 Experiment: 2106900 HV2 Pre
 Assay Conditions:

Study Code: ICCR 2106900
 Date Plated: 28.05.2020
 Date Counted: 04.06.2020

Without metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 pKM101	Isocycloseram/	33 µg	265.7	11.2	1.0	263, 278, 256
	Emamectin	100 µg	273.3	5.7	1.0	278, 275, 267
	Benzoate SC	333 µg	249.3	12.5	1.0	249, 262, 237
	(A23220A)	1000 µg	272.3	7.6	1.0	274, 279, 264
		2500 µg	262.3	19.1	1.0	280, 265, 242
		5000 µg	266.3	7.6	1.0	258, 268, 273
	Deionised water		262.3	5.7		256, 264, 267
	Untreated		252.0	14.8		269, 242, 245
WP2 uvrA pKM101	Isocycloseram/	33 µg	335.0	28.2	1.0	303, 346, 356
	Emamectin	100 µg	364.3	30.2	1.1	331, 390, 372
	Benzoate SC	333 µg	330.0	7.9	1.0	321, 333, 336
	(A23220A)	1000 µg	316.3	13.6	1.0	301, 321, 327
		2500 µg	288.7	16.2	0.9	298, 298, 270
		5000 µg	297.0	5.3	0.9	293, 295, 303
	Deionised water		325.0	21.5		304, 324, 347
	Untreated		301.0	3.5		299, 299, 305
TA 1535	NaN3	10 µg	1102.0	90.1	118.1	1005, 1118, 1183
TA 1537	4-NOPD	50 µg	91.7	8.6	8.9	90, 84, 101
TA 98	4-NOPD	10 µg	939.3	80.6	37.6	851, 1009, 958
TA 100	NaN3	10 µg	1857.7	170.0	18.5	2026, 1861, 1686
WP2 pKM101	MMS	2.0 µL	2947.0	609.8	11.2	2394, 2846, 3601
WP2 uvrA pKM101	MMS	2.0 µL	3187.0	36.0	9.8	3187, 3151, 3223

Key to Positive Controls

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

TABLE 6 Experiment II: 2106900 HV2 Pre Incubation With Metabolic Activation

Study Name: 2106900
Experiment: 2106900 HV2 Pre
Assay Conditions:

Study Code: ICCR 2106900
Date Plated: 28.05.2020
Date Counted: 04.06.2020

With metabolic activation						
Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Isocycloseram/	33 µg	13.7	2.3	1.0	15, 11, 15
	Emamectin	100 µg	12.7	3.2	0.9	14, 9, 15
	Benzoate SC	333 µg	14.0	1.7	1.0	15, 15, 12
	(A23220A)	1000 µg	13.3	2.3	1.0	16, 12, 12
		2500 µg	15.0	2.6	1.1	17, 16, 12
		5000 µg	13.3	1.2	1.0	14, 14, 12
	Deionised water		13.7	3.2		15, 16, 10
	Untreated		17.3	4.2		16, 22, 14
TA 1537	Isocycloseram/	33 µg	17.3	2.9	1.1	19, 19, 14
	Emamectin	100 µg	19.7	0.6	1.2	20, 20, 19
	Benzoate SC	333 µg	16.0	3.6	1.0	12, 17, 19
	(A23220A)	1000 µg	13.3	2.1	0.8	11, 15, 14
		2500 µg	15.7	4.5	1.0	11, 16, 20
		5000 µg	15.0	3.6	0.9	12, 19, 14
	Deionised water		16.3	4.0		20, 12, 17
	Untreated		15.0	4.0		19, 15, 11
TA 98	Isocycloseram/	33 µg	43.3	3.1	1.2	44, 40, 46
	Emamectin	100 µg	45.7	3.5	1.3	49, 46, 42
	Benzoate SC	333 µg	41.7	9.0	1.2	52, 37, 36
	(A23220A)	1000 µg	45.0	11.5	1.3	56, 33, 46
		2500 µg	28.3	8.5	0.8	37, 20, 28
		5000 µg	36.0	4.6	1.0	37, 40, 31
	Deionised water		35.7	1.2		35, 37, 35
	Untreated		37.0	1.0		37, 38, 36
TA 100	Isocycloseram/	33 µg	98.7	25.5	1.0	82, 86, 128
	Emamectin	100 µg	118.3	21.2	1.2	142, 112, 101
	Benzoate SC	333 µg	130.3	22.5	1.3	152, 132, 107
	(A23220A)	1000 µg	132.7	5.5	1.3	138, 133, 127
		2500 µg	131.7	10.0	1.3	142, 131, 122
		5000 µg	97.7	6.7	1.0	101, 90, 102
	Deionised water		100.7	22.9		86, 127, 89
	Untreated		98.7	10.8		94, 91, 111

Study Name: 2106900
 Experiment: 2106900 HV2 Pre
 Assay Conditions:

Study Code: ICCR 2106900
 Date Plated: 28.05.2020
 Date Counted: 04.06.2020

With metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 pKM101	Isocycloseram/	33 µg	279.0	38.0	0.9	321, 269, 247
	Emamectin	100 µg	270.3	17.2	0.9	263, 258, 290
	Benzoate SC	333 µg	257.7	18.8	0.9	241, 254, 278
	(A23220A)	1000 µg	312.7	12.1	1.0	322, 299, 317
		2500 µg	301.7	37.0	1.0	338, 303, 264
		5000 µg	314.0	39.7	1.1	270, 325, 347
	Deionised water		298.3	15.3		289, 290, 316
	Untreated		304.0	9.5		310, 309, 293
WP2 uvrA pKM101	Isocycloseram/	33 µg	374.7	16.2	1.1	384, 384, 356
	Emamectin	100 µg	364.0	10.4	1.1	352, 370, 370
	Benzoate SC	333 µg	386.3	29.3	1.2	408, 353, 398
	(A23220A)	1000 µg	390.0	16.5	1.2	409, 382, 379
		2500 µg	380.0	2.0	1.1	380, 378, 382
		5000 µg	368.0	22.6	1.1	362, 349, 393
	Deionised water		330.7	31.7		364, 301, 327
	Untreated		372.7	17.9		377, 388, 353
TA 1535	2-AA	2.5 µg	289.0	6.2	21.1	291, 294, 282
TA 1537	2-AA	2.5 µg	349.3	35.7	21.4	362, 309, 377
TA 98	2-AA	2.5 µg	3518.3	298.5	98.6	3347, 3345, 3863
TA 100	2-AA	2.5 µg	2318.3	230.5	23.0	2520, 2368, 2067
WP2 pKM101	2-AA	10.0 µg	1037.0	20.7	3.5	1020, 1031, 1060
WP2 uvrA pKM101	2-AA	10.0 µg	1738.7	72.9	5.3	1656, 1794, 1766

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 November 2016 until August 2018 representing approx. 600 experiments (WP2 pKM101, WP2 uvrA pKM101 the historical data are based on approx. 60 experiments).

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

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	11	2.3	6	22	12	2.3	7	22
	Untreated control	11	2.9	6	28	12	2.8	7	23
	Positive control	1245	161.4	367	1791	398	61.0	183	613
TA 1537	Solvent control	10	2.2	6	19	13	3.2	7	30
	Untreated control	10	2.7	5	21	14	3.6	6	29
	Positive control	94	30.0	48	231	170	64.8	81	421
TA 98	Solvent control	26	4.2	13	43	36	6.1	12	56
	Untreated control	27	4.7	14	40	39	6.4	12	59
	Positive control	421	176.8	196	2068	3908	815.0	223	5918
TA 100	Solvent control	160	29.3	79	214	148	31.3	76	216
	Untreated control	181	26.1	80	235	171	27.7	87	218
	Positive control	2074	262.7	511	2850	3626	981.9	553	5860
WP2 pKM101	Solvent control	205	27.6	171	289	233	29.3	194	310
	Untreated control	220	32.2	167	297	259	33.4	204	338
	Positive control	3894	522.5	2576	5458	1277	403.5	950	4063
WP2 uvrA pKM101	Solvent control	324	39.5	243	389	378	48.0	277	469
	Untreated control	339	32.3	279	402	389	39.2	302	476
	Positive control	3706	589.3	2754	5261	2191	252.7	1720	2731

Mean = mean value of revertants/plate

SD = standard deviation

Min = minimal value

Max = maximal value

APPENDIX 2 Copy of GLP Certificate

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

HESSEN



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 address)

Prüfungen nach Kategorien/Areas of Expertise

(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
8 Analytische Prüfungen an biologischen Materialien

2 Toxicity studies
3 Mutagenicity studies
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

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,
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, Energy, Agriculture and Consumer Protection; Department II 10; P.O. Box 31 09; 65189 Wiesbaden
Translation of stamp inscription:
Hessian Ministry for Environment, Rural Regions and Consumer Protection

APPENDIX 3 Certificate of S9



CERTIFICATE

ICCR-Roßdorf S9 Preparation Lot No. 281119B

Date of preparation: November 28, 2019

Release date: December 10, 2019

Protein assay: 29.7 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	25
control	100	44
10 µg/plate	0	44
2-Aminoanthracene	100	2058
10 µg/plate	0	34
Benzo(a)pyrene	100	113

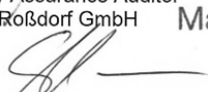
The S9 was obtained from the livers of male Wistar rats which received triple treatments of 80 mg / kg body weight Phenobarbital and β -Naphthoflavone orally on consecutive days. The livers were prepared 24 hours after the last treatment.


Quality Assurance Auditor
ICCR-Roßdorf GmbH

Marina Hahn

18. DEZ. 2019

Date


Dr. Steffen Naumann
Study Director
ICCR-Roßdorf GmbH

18. DEZ. 2019

Date

ICCR-Roßdorf GmbH
In den Leppsteinswiesen 19, 64380 Roßdorf, Deutschland
T +49 6154 8070 F +49 6154 83399
Registergericht Darmstadt, HRB 6837, USt.-ID DE812333696
Geschäftsführer: Dr. Markus Schulz

SOP Origin TS-SOP S9_21

APPENDIX 4 Certificate of Analysis



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

Certificate of Analysis

A23220A isocycloseram/emamectin benzoate SC (200/050) TSC002-041-001

Batch Identification	TSC002-041-001
Other Batch ID	1122866
Product Code	A23220A
Other Product Code(s)	isocycloseram/emamectin benzoate SC (200/050)

Chemical Analysis
(Active Ingredient content)

- | | |
|---|--------------------------------------|
| - Identity of the Active Ingredient(s)* | confirmed |
| - Content of isocycloseram* | 17.5 % w/w corresponding to 201 g/l |
| - Content of emamectin benzoate* | 4.18 % w/w corresponding to 48.1 g/l |
- The Active Ingredient(s) content is within the FAO limits.

Methodology used for Characterization / Recertification	LC, chiral LC, oscillating density meter
---	--

Physical Analysis

- | | |
|--------------|------------------------|
| - Appearance | brown liquid |
| - Density* | 1150 kg/m ³ |

Stability:

- | | |
|------------------------|---------------------|
| - Storage Temperature | < 30°C |
| - Recertification Date | End of January 2023 |

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

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

Study number of batch characterization: CHMU200180

Study number(s) of batch recertification:

Authorization:

19-Feb-2020

Dr. Karine Heintz
Analytical Development & Product Chemistry