

Pinoxaden/Cloquintocet-Mexyl
Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) - *Salmonella*
***Typhimurium* and *Escherichia Coli* Reverse Mutation Assay**

Final Report

DATA REQUIREMENT(S): OECD 471 (1997)

AUTHOR(S): Dr. Steffi Chang

STUDY COMPLETION DATE: 04 August 2016

PERFORMING LABORATORY: Envigo CRS GmbH
In den Leppsteinswiesen 19
64380 Rossdorf, Germany

LABORATORY PROJECT ID: Report Number: 1768500
Study Number: 1768500
Task Number: TK0279306

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

VOLUME 1 OF 1 OF STUDY

PAGE 1 OF 39

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

This page is intentionally left blank.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study performed in the test facility of Envigo CRS 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 [C(97)186/Final]
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 (MHLW, 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: 04 August 2016

Performing Laboratory:
Envigo CRS GmbH
In den Leppsteinswiesen 19
64380 Rossdorf, Germany

FLAGGING STATEMENT

This page is intentionally left blank.

QUALITY ASSURANCE STATEMENT

Envigo Study Number: 1768500
Test substance: Pinoxaden/Cloquintocet-Mexyl EC (A13617AV)
Study director: Dr. Steffi Chang
Study Title: Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) -
Salmonella Typhimurium and
Escherichia Coli Reverse Mutation Assay

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

The GSP471.Ames.Syngenta.03 was reviewed for compliance on 18 April 2016.

Type of Inspection	Date(s) of Inspection	Date Reporting to Study Director, Test Facility Management
Study Plan Verification	12 May 2016	12 May 2016
Amendment to study plan 1	21 July 2016	21 July 2016
Process – based Test performance	10 June 2016	10 June 2016
Report Audit	13 July 2016	13 July 2016

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.



Marina Hahn

Quality Assurance Auditor
Envigo CRS GmbH

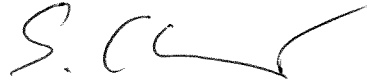
04 August 2016

Date

PROJECT STAFF SIGNATURE

Study Director

Dr. Steffi Chang

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

Date: 04 August 2016

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Dr. Steffi Chang	Study Director
Dr. Hans-Eric Wollny	Deputy Study Director
Dr. Wolfgang Völkner	Management
Frauke Hermann	Head of Quality Assurance Unit
Priscila Fagundes	Syngenta Study Manager

Study Dates

Study initiation date:	12 May 2016
Experimental start date:	24 May 2016
Experimental completion date:	20 June 2016

Deviations from the Guidelines

None

Retention of Samples

Raw data and a sample of the test substance.

Performing Laboratory Test Substance Reference Number

S1812311

Other

Envigo CRS will archive:

Records and documentation relating to this study will be maintained in the archives of Envigo CRS GmbH for a period of 4 years from the date on which the Study Director signs the final report. This will include raw data, specimens, and a sample of the test item that support the reconstruction of the study. Specimens that no longer afford evaluation will be discarded in accordance with Standard Operating Procedures and without further notice.

At termination of the aforementioned period, the records and documentation will be transferred to the GLP compliant Archive of Envigo CRS (Switzerland) Ltd. at Füllinsdorf, Switzerland, for further archiving up to a total archiving period of 15 years.

Envigo will retain the study plan, final report and any amendments indefinitely.

Deviations from the study plan

There were no deviations from study plan.

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 Regulatory Guidelines.....	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 Envigo CRS)	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.....	19
3.8.1 Data recording.....	19

3.8.2	Acceptability of the assay	19
3.8.3	Evaluation of results.....	19
3.8.4	Biometry.....	19
3.9	Major Computerized Systems	20
4.0	RESULTS AND DISCUSSION	21
4.1	Concentration Selection	21
4.2	Discussion	21
5.0	CONCLUSIONS	22
6.0	REFERENCES	23
TABLES SECTION		24
TABLE 1	Summary of Results Pre-Experiment/Experiment I.....	25
TABLE 2	Summary of Results Experiment II.....	26
TABLE 3	Pre-Experiment and Experiment I: 1768500 VV Plate Incorporation.....	27
TABLE 4	Pre-Experiment and Experiment I: 1768500 VV Plate Incorporation.....	29
TABLE 5	Experiment II: 1768500 HV2 Pre Incubation	31
TABLE 6	Experiment II: 1768500 HV2 Pre Incubation	33
TABLE 7	Batch Control of S9.....	35
APPENDICES SECTION		36
APPENDIX 1	Historical Control Data	37
APPENDIX 2	Copy of GLP Certificate	38
APPENDIX 3	Certificate of Analysis.....	39

1.0 EXECUTIVE SUMMARY

1.1 Study Design

This study was performed to investigate the potential of Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, and the *Escherichia coli* strains WP2 *uvrA* pKM101 and WP2 pKM101.

1.2 Results

The plates incubated with the test item showed reduced background growth in strains TA 1537, TA 98 and TA 100.

Cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5) were observed in strain TA 100 with and without metabolic activation (S9 mix) and in strain WP2 pKM101 without metabolic activation (S9 mix). No cytotoxic effects occurred in the remaining strains with and without metabolic activation.

A minor increase in revertant colony numbers following treatment with Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) was observed in experiment I in strain TA 1537 with S9 mix, but the threshold of twice the number of the corresponding solvent control was not reached.

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

1.3 Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test substance did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) is considered to be non-mutagenic in this *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⁻ → his⁺ and trp⁻ → trp⁺ reversions, respectively. The *S. typhimurium* and *Escherichia 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 Regulatory Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines:

“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:	Pinoxaden/Cloquintocet-Mexyl EC (A13617AV)
Product Code:	A13617AV
Batch:	SMO3K0051
Content of pinoxaden:	5.23 % w/w corresponding to 50.5 g/L
Content of cloquintocet-mexyl:	1.35 % w/w corresponding to 13.0 g/L
Appearance:	Amber liquid
Recertification Date:	31 December 2016
Storage Conditions:	At room temperature
Stability in Solvent:	Not indicated by the Sponsor

Concentrations were not adjusted to the content of the active ingredients.

On the day of the experiment, the test substance Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) was suspended in DMF (purity \geq 99 %). The solvent was chosen because of its solubilisation properties and its relative non-toxicity to the bacteria (2).

3.2 Controls

3.2.1 Negative controls

Concurrent untreated and solvent controls were performed.

3.2.2 Positive control substances

Without metabolic activation

Strains:	TA1535, TA100
Name:	sodium azide, NaN_3
Supplier:	SERVA, 69042 Heidelberg/Germany
Batch No.:	070324
Purity:	at least 99 %
Dissolved in:	deionised water
Concentration:	10 $\mu\text{g}/\text{plate}$
Strains:	TA1537, TA98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	Sigma Aldrich, 82024 Taufkirchen/Germany
Batch No.:	MKBM 5257V
Purity:	> 99.9 %
Dissolved in:	DMSO (purity >99 %, Fisher Leics LE11 5RG)
Concentration:	10 $\mu\text{g}/\text{plate}$ in strain TA 98, 50 $\mu\text{g}/\text{plate}$ in strain TA 1537
Strains:	WP2 <i>uvrA</i> (pKM101), WP2 (pKM101)
Name:	methyl methane sulfonate, MMS
Supplier:	Sigma Aldrich, 82024 Taufkirchen/Germany
Batch No.:	MKBJ 8702V
Purity:	> 99.0 %
Dissolved in:	deionised water
Concentration:	2.0 $\mu\text{L}/\text{plate}$

With metabolic activation

Strains:	TA1535, TA1537, TA98, TA100, WP2 <i>uvrA</i> (pKM101), WP2 (pKM 101)
Name:	2-aminoanthracene, 2-AA
Supplier:	Sigma Aldrich, 82024 Taufkirchen/Germany
Batch No.:	STBD 3302V
Purity:	97.5 %
Dissolved in:	DMSO (purity > 99 %, Fisher Leics LE11 5RG)
Concentration:	2.5 $\mu\text{g}/\text{plate}$ (TA1535, TA1537, TA98, TA100), 10 $\mu\text{g}/\text{plate}$ (WP2 <i>uvrA</i> (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 latter 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 TA 98 and TA 100 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 TA 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 Envigo CRS 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 WP2pKM101 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 (MERCK, D-64293 Darmstadt) in liquid nitrogen.

3.3.3 Precultures

The thawed bacterial suspension was transferred into 250 mL Erlenmeyer flasks containing 50 mL nutrient medium. A solution of 50 μ L ampicillin (25 μ g/mL) was added to the strains TA 98, TA 100, WP2 *uvrA* pKM101, and WP2 pKM101. This nutrient medium contains per liter:

8 g Nutrient Broth (MERCK, D-64293 Darmstadt)

5 g NaCl (MERCK, D-64293 Darmstadt)

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 \times HCl \times H₂O*

12.2 mg Biotin*

* (MERCK, D-64293 Darmstadt)

for *Escherichia coli*:

7.0 g Agar Agar*

6.0 g NaCl*

10.2 mg Tryptophan*

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 Envigo CRS)

Phenobarbital/ β -naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from 8 – 12 weeks old 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 9000g. 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 was routinely tested with 2-aminoanthracene as well as benzo[a]pyrene (Table 7).

The protein concentration in the S9 preparation was 30.7 mg/mL (lot no. 140116B) 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 are 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 were the same as described below for experiment I (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 under “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 slight cytotoxic effects were observed in experiment I at least six concentrations were tested in experiment II. 5000 µg/plate was chosen as the maximal concentration.

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

Strains TA 1535 and TA 100: 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

The remaining strains: 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 100 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer* and 100 µL bacteria suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (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 in the dark, plates were then stored at 4°C until counted. (6).

In parallel to each test a sterile control of the test item was performed and documented in the raw data. Therefore, 100 µL of the stock solution, 500 µL S9 mix / S9 mix substitution buffer were mixed with 2.0 mL overlay agar 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 validated computer system (cf. 3.9, Major computerized systems), which was connected to a PC with printer to print out the individual values and the means from the plates for each concentration together with standard deviations and ratios of revertants compared to the solvent control (see tables of results). Due to precipitation of the test substance the colonies were partly counted manually.

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 a significant increase in mutant colony frequencies
- 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 exceeding the threshold of twice the colony count of the corresponding solvent control is observed (1).

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

An increase exceeding 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 Systems

Petri Viewer (Sorcerer Colony Counter v3.0 Perceptive Instruments Ltd, Suffolk CB9 7BN, UK) with the software program Ames Study Manager (v1.24).

4.0 RESULTS AND DISCUSSION

4.1 Concentration Selection

In the pre-experiment the concentration range of the test substance was 3 - 5000 µg/plate. The pre-experiment is reported as experiment I. Based on the observed cytotoxicity of the test substance at least seven concentrations were tested in experiment II, 5000 µg/plate was chosen as the maximal concentration.

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

Strains TA 1535 and TA 100: 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

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

4.2 Discussion

The test substance Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) 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 *uvrA* pKM101 and WP2 pKM101.

The assay was performed with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test substance was tested at the following concentrations:

Experiment I: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II:

Strains TA 1535 and TA 100: 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

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

The test substance precipitated in the overlay agar in the test tubes from 1000 to 5000 µg/plate in experiment I and from 2500 to 5000 µg/plate in experiment II. Precipitation of the test substance in the overlay agar on the incubated agar plates was observed in experiment I at 5000 µg/plate without S9 mix and from 2500 to 5000 µg/plate with S9 mix and in experiment II at 5000 µg/plate with and without S9 mix. The undissolved particles had no influence on the data recording.

The plates incubated with the test substance showed reduced background growth at the following concentrations ($\mu\text{g}/\text{plate}$):

Strain	Experiment I		Experiment II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA 1535	/	/	/	/
TA 1537	/	1000 – 5000	5000	5000
TA 98	/	5000	/	/
TA 100	/	2500 – 5000	/	5000
WP2 pKM101	/	/	/	/
WP2 <i>uvrA</i> pKM101	/	/	/	/

/ = normal background growth

Cytotoxic effects, evident as a reduction in the number of revertants (below the induction factor of 0.5), were observed at the following concentrations ($\mu\text{g}/\text{plate}$):

Strain	Experiment I		Experiment II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA 1535	/	/	/	/
TA 1537	/	/	/	/
TA 98	/	/	/	/
TA 100	5000	5000	1000 – 5000	2500 – 5000
WP2 pKM101	/	/	2500 – 5000	/
WP2 <i>uvrA</i> pKM101	/	/	/	/

/ = No cytotoxic effects, evident as a reduction in the number of revertants (below the induction factor of 0.5)

A minor increase in revertant colony numbers following treatment with Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) was observed in experiment I in strain TA 1537 with S9 mix, but the threshold of twice the number of the corresponding solvent control was not reached and the fold value decreased at higher concentration. Furthermore, all increased colony counts remained well within the historical range of the negative or solvent controls. It was judged that this effect is caused by biological fluctuations at such low numbers of colonies and does not indicate a true mutagenic potential.

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

5.0 CONCLUSIONS

During the described mutagenicity tests and under the experimental conditions reported, Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, Pinoxaden/Cloquintocet-Mexyl EC (A13617AV) is considered to be non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

6.0 REFERENCES

1. Hollstein, M., J. McCann, F.A. Angelosanto, and W.W. Nichols (1979)
Short-term tests for carcinogens and mutagens
Mutation Res. 65, 133-226
2. Maron D.M., J. Katzenellenbogen, and B.N. Ames (1981)
Compatibility of organic solvents with the Salmonella/Microsome Test
Mutation Res. 88, 343-350
3. Maron D.M., Ames, B.N. (1983)
Revised methods for the Salmonella mutagenicity test
Mutation Res. 113, 173-215
4. Green, M.H.L. and W.J. Muriel (1976)
Mutagen Testing Using TRP⁺ Reversion in Escherichia Coli
Mutation. Res. 38, 3- 32
5. Ames, B.N., J. McCann, and E. Yamasaki (1977)
Methods for detecting carcinogens and mutagens with the Salmonella/mammalian
microsome mutagenicity test
In: B.J. Kilbey et al. (Eds.) "Handbook of Mutagenicity Test Procedures" Elsevier,
Amsterdam, 1-17
6. de Serres F.J. and M.D. Shelby (1979)
Recommendations on data production and analysis using the Salmonella/microsome
mutagenicity assay
Mutation Res. 64, 159-165
7. Mortelmans K., Riccio ES.(2000)
The bacterial tryptophan reverse mutation assay with Escherichia coli WP2
Mutation Res. 455 61-69

TABLES SECTION

TABLE 1 Summary of Results Pre-Experiment/Experiment I

Study Name: 1768500
 Experiment: 1768500 VV Plate
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 24.05.2016
 Date Counted: 31.05.2016

Metabolic Activation	Test Group	Concentration (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 <i>uvrA</i> pKM101
Without Activation	DMF		13 ± 3	13 ± 5	25 ± 6	163 ± 27	230 ± 8	351 ± 29
	Untreated		10 ± 1	12 ± 5	33 ± 4	183 ± 15	248 ± 12	344 ± 47
	Pinoxaden/	3 µg	14 ± 7	13 ± 2	27 ± 5	191 ± 8	215 ± 20	363 ± 26
	Cloquintocet-	10 µg	10 ± 2	13 ± 5	30 ± 5	188 ± 5	207 ± 7	368 ± 16
	Mexyl EC	33 µg	12 ± 3	10 ± 2	30 ± 2	194 ± 5	226 ± 5	357 ± 1
	(A13617AV)	100 µg	16 ± 4	12 ± 1	32 ± 9	207 ± 22	229 ± 4	357 ± 13
		333 µg	12 ± 6	10 ± 5	29 ± 7	194 ± 11	243 ± 33	379 ± 17
		1000 µg	16 ± 1	8 ± 5	26 ± 6	163 ± 14	204 ± 5	368 ± 2
		2500 µg	8 ± 2	8 ± 2	25 ± 3	97 ± 8	197 ± 12	342 ± 10
		5000 µg	9 ± 5 ^P	11 ± 4 ^P	22 ± 1 ^P	50 ± 5 ^P	198 ± 35 ^P	337 ± 20 ^P
	NaN3	10 µg	1463 ± 44			2063 ± 196		
	4-NOPD	10 µg			393 ± 27			
	4-NOPD	50 µg		67 ± 3				
	MMS	2.0 µL					4200 ± 191	4400 ± 416
With Activation	DMF		13 ± 2	11 ± 3	37 ± 3	194 ± 19	240 ± 9	413 ± 21
	Untreated		18 ± 6	22 ± 1	45 ± 9	202 ± 18	281 ± 10	444 ± 24
	Pinoxaden/	3 µg	13 ± 5	13 ± 2	38 ± 10	158 ± 39	268 ± 24	398 ± 7
	Cloquintocet-	10 µg	10 ± 0	14 ± 4	44 ± 6	139 ± 15	248 ± 32	406 ± 4
	Mexyl EC	33 µg	10 ± 4	13 ± 4	35 ± 5	154 ± 16	215 ± 22	401 ± 19
	(A13617AV)	100 µg	11 ± 5	14 ± 3	34 ± 1	182 ± 27	274 ± 33	398 ± 31
		333 µg	12 ± 6	15 ± 4	35 ± 10	193 ± 24	238 ± 17	396 ± 27
		1000 µg	9 ± 5	18 ± 3 ^R	38 ± 5	173 ± 12	210 ± 9	391 ± 48
		2500 µg	12 ± 5 ^P	21 ± 3 ^{PR}	28 ± 8 ^P	125 ± 23 ^{PR}	185 ± 20 ^P	352 ± 59 ^P
		5000 µg	6 ± 1 ^P	15 ± 3 ^{PR}	29 ± 3 ^{PR}	81 ± 1 ^{PR}	204 ± 36 ^P	370 ± 34 ^P
	2-AA	2.5 µg	432 ± 7	237 ± 12	4348 ± 548	5716 ± 131		
2-AA	10.0 µg					1431 ± 38	2103 ± 33	

Key to Positive Controls

Key to Plate Postfix Codes

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

P Precipitate
 R Reduced background growth

TABLE 2 Summary of Results Experiment II

Study Name: 1768500
 Experiment: 1768500 HV2 Pre
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 17.06.2016
 Date Counted: 20.06.2016

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	DMF Untreated		11 ± 1	9 ± 3	26 ± 6	164 ± 22	217 ± 8	337 ± 16	
	Pinoxaden/ Cloquintocet-Mexyl EC (A13617AV)	10 µg	12 ± 3	7 ± 3	28 ± 10	201 ± 1	235 ± 13	315 ± 29	
		33 µg	10 ± 3			157 ± 12			
		100 µg	11 ± 1	7 ± 3	24 ± 5	178 ± 17	208 ± 4	326 ± 7	
		333 µg	12 ± 3	10 ± 5	23 ± 3	172 ± 11	222 ± 7	339 ± 14	
		1000 µg	10 ± 1	8 ± 2	22 ± 1	109 ± 24	210 ± 19	318 ± 9	
		2500 µg	11 ± 4	7 ± 3	25 ± 3	71 ± 15	118 ± 17	272 ± 49	
		5000 µg	11 ± 2	8 ± 2	22 ± 2	60 ± 4	72 ± 4	190 ± 3	
		NaN3	10 µg	11 ± 1 ^P	7 ± 3 ^{PR}	20 ± 1 ^P	48 ± 9 ^P	61 ± 9 ^P	161 ± 21 ^P
		4-NOPD	10 µg	1179 ± 27			1891 ± 24		
		4-NOPD	50 µg		67 ± 13	364 ± 5			
	MMS	2.0 µL				3725 ± 197	2830 ± 106		
With Activation	DMF Untreated		16 ± 6	13 ± 3	40 ± 9	187 ± 23	267 ± 14	407 ± 10	
	Pinoxaden/ Cloquintocet-Mexyl EC (A13617AV)	10 µg	11 ± 1	12 ± 3	38 ± 3	211 ± 12	282 ± 14	406 ± 15	
		33 µg	15 ± 1			173 ± 16			
		100 µg	16 ± 8	14 ± 5	40 ± 2	168 ± 9	241 ± 20	403 ± 3	
		333 µg	14 ± 3	16 ± 6	36 ± 10	175 ± 22	256 ± 9	391 ± 17	
		1000 µg	12 ± 3	17 ± 6	40 ± 5	171 ± 15	224 ± 12	382 ± 15	
		2500 µg	14 ± 5	14 ± 2	43 ± 13	91 ± 21	242 ± 22	398 ± 10	
		5000 µg	14 ± 4	18 ± 6	26 ± 8	63 ± 9	177 ± 10	376 ± 7	
		2-AA	2.5 µg	13 ± 3 ^P	10 ± 2 ^{PRM}	29 ± 5 ^P	41 ± 14 ^{PR}	147 ± 6 ^P	349 ± 23 ^P
		2-AA	10.0 µg	399 ± 27	219 ± 26	5167 ± 668	5103 ± 35	1201 ± 24	2253 ± 75

Key to Positive Controls

Key to Plate Postfix Codes

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

P Precipitate
 R Reduced background growth
 M Manual count

TABLE 3 Pre-Experiment and Experiment I: 1768500 VV Plate Incorporation

Study Name: 1768500
 Experiment: 1768500 VV Plate
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 24.05.2016
 Date Counted: 31.05.2016

Without metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Pinoxaden/ - Cloquintocet Mexyl EC (A13617AV)	3 µg	13.7	7.2	1.1	22, 10, 9
		10 µg	10.0	1.7	0.8	9, 12, 9
		33 µg	12.0	2.6	0.9	10, 15, 11
		100 µg	16.0	3.6	1.2	12, 19, 17
		333 µg	12.3	5.5	0.9	16, 15, 6
		1000 µg	15.7	1.2	1.2	15, 17, 15
		2500 µg	8.0	1.7	0.6	7, 10, 7
		5000 µg	8.7	4.6	0.7	6 P, 6 P, 14 P
		DMF		13.0	3.5	
	Untreated Control		9.7	1.2		11, 9, 9
TA 1537	Pinoxaden/ - Cloquintocet Mexyl EC (A13617AV)	3 µg	13.3	2.1	1.1	11, 15, 14
		10 µg	13.0	5.2	1.0	16, 16, 7
		33 µg	10.3	1.5	0.8	12, 10, 9
		100 µg	11.7	0.6	0.9	12, 12, 11
		333 µg	10.3	4.7	0.8	14, 5, 12
		1000 µg	8.3	4.9	0.7	6, 14, 5
		2500 µg	8.3	2.3	0.7	7, 11, 7
		5000 µg	10.7	4.0	0.8	10 P, 7 P, 15 P
		DMF		12.7	4.9	
	Untreated Control		11.7	4.9		6, 15, 14
TA 98	Pinoxaden/ - Cloquintocet Mexyl EC (A13617AV)	3 µg	27.0	5.2	1.1	30, 30, 21
		10 µg	30.0	5.0	1.2	25, 30, 35
		33 µg	30.0	2.0	1.2	28, 30, 32
		100 µg	32.3	9.0	1.3	41, 23, 33
		333 µg	28.7	7.4	1.1	23, 26, 37
		1000 µg	25.7	5.5	1.0	31, 26, 20
		2500 µg	25.3	2.9	1.0	22, 27, 27
		5000 µg	21.7	0.6	0.9	22 P, 21 P, 22 P
		DMF		25.3	6.0	
	Untreated Control		33.3	3.5		33, 30, 37
TA 100	Pinoxaden/ - Cloquintocet Mexyl EC (A13617AV)	3 µg	191.3	8.1	1.2	190, 200, 184
		10 µg	188.3	5.1	1.2	194, 187, 184
		33 µg	194.0	5.2	1.2	188, 197, 197
		100 µg	206.7	21.8	1.3	226, 211, 183
		333 µg	194.0	11.4	1.2	202, 199, 181
		1000 µg	162.7	14.0	1.0	167, 147, 174
		2500 µg	97.0	7.9	0.6	106, 94, 91
		5000 µg	50.0	4.6	0.3	54 P, 51 P, 45 P
		DMF		163.0	26.5	
	Untreated Control		183.0	14.8		166, 193, 190

Key to Plate Postfix Codes

P Precipitate

TABLE 3 Pre-Experiment and Experiment I: 1768500 VV Plate Incorporation (Continued)

Study Name: 1768500
 Experiment: 1768500 VV Plate
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 24.05.2016
 Date Counted: 31.05.2016

Without metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 pKM101	Pinoxaden/ -	3 µg	214.7	19.6	0.9	220, 231, 193
		10 µg	207.3	6.7	0.9	204, 203, 215
	Cloquintocet Mexyl EC (A13617AV)	33 µg	225.7	4.6	1.0	223, 231, 223
		100 µg	229.0	3.6	1.0	230, 232, 225
		333 µg	243.0	33.3	1.1	257, 205, 267
		1000 µg	204.0	5.3	0.9	206, 198, 208
		2500 µg	197.0	12.0	0.9	197, 209, 185
		5000 µg	198.3	35.0	0.9	210 P, 226 P, 159 P
	DMF		230.0	7.8		239, 225, 226
	Untreated Control		248.0	12.3		234, 253, 257
WP2 uvrA pKM101	Pinoxaden/ -	3 µg	363.0	26.2	1.0	339, 391, 359
		10 µg	367.7	16.3	1.0	362, 355, 386
	Cloquintocet Mexyl EC (A13617AV)	33 µg	356.7	0.6	1.0	357, 357, 356
		100 µg	356.7	13.3	1.0	349, 349, 372
		333 µg	379.3	16.5	1.1	393, 384, 361
		1000 µg	368.3	1.5	1.0	367, 370, 368
		2500 µg	342.0	9.5	1.0	352, 333, 341
		5000 µg	337.3	20.4	1.0	342 P, 315 P, 355 P
	DMF		351.3	28.9		366, 318, 370
	Untreated Control		343.7	46.7		360, 291, 380
TA 1535	NaN3	10 µg	1462.7	44.1	112.5	1412, 1492, 1484
TA 1537	4-NOPD	50 µg	66.7	2.5	5.3	69, 64, 67
TA 98	4-NOPD	10 µg	393.0	27.4	15.5	414, 403, 362
TA 100	NaN3	10 µg	2063.3	195.6	12.7	1990, 1915, 2285
WP2 pKM101	MMS	2.0 µL	4200.3	190.9	18.3	4316, 4305, 3980
WP2 uvrA pKM101	MMS	2.0 µL	4400.3	416.2	12.5	4249, 4081, 4871

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 and Experiment I: 1768500 VV Plate Incorporation

Study Name: 1768500
 Experiment: 1768500 VV Plate
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 24.05.2016
 Date Counted: 31.05.2016

With metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Pinoxaden/	3 µg	13.3	5.1	1.1	19, 12, 9
	Cloquintocet-	10 µg	10.0	0.0	0.8	10, 10, 10
	Mexyl EC	33 µg	10.3	4.0	0.8	11, 6, 14
	(A13617AV)	100 µg	11.0	5.0	0.9	6, 11, 16
		333 µg	12.0	6.1	0.9	16, 15, 5
		1000 µg	9.3	4.9	0.7	15, 6, 7
		2500 µg	11.7	4.9	0.9	14 P, 15 P, 6 P
		5000 µg	6.3	0.6	0.5	7 P, 6 P, 6 P
	DMF		12.7	2.3		14, 10, 14
	Untreated Control		18.3	6.4		11, 21, 23
TA 1537	Pinoxaden/	3 µg	13.3	2.1	1.2	14, 15, 11
	Cloquintocet-	10 µg	14.0	4.4	1.2	9, 16, 17
	Mexyl EC	33 µg	12.7	4.0	1.1	9, 17, 12
	(A13617AV)	100 µg	14.0	2.6	1.2	15, 11, 16
		333 µg	15.3	4.2	1.4	14, 12, 20
		1000 µg	17.7	3.1	1.6	15 R, 21 R, 17 R
		2500 µg	20.7	3.2	1.8	23 P R, 17 P R, 22 P R
		5000 µg	14.7	3.2	1.3	17 P R, 11 P R, 16 P R
	DMF		11.3	3.2		15, 9, 10
	Untreated Control		21.7	0.6		21, 22, 22
TA 98	Pinoxaden/	3 µg	38.3	10.3	1.0	27, 41, 47
	Cloquintocet-	10 µg	44.3	5.9	1.2	51, 42, 40
	Mexyl EC	33 µg	34.7	4.7	0.9	33, 40, 31
	(A13617AV)	100 µg	34.3	1.2	0.9	33, 35, 35
		333 µg	35.3	9.5	1.0	35, 45, 26
		1000 µg	37.7	5.0	1.0	33, 37, 43
		2500 µg	28.3	8.1	0.8	27 P, 37 P, 21 P
		5000 µg	29.3	2.9	0.8	31 P R, 31 P R, 26 P R
	DMF		36.7	2.9		40, 35, 35
	Untreated Control		44.7	8.7		52, 35, 47
TA 100	Pinoxaden/	3 µg	158.0	39.1	0.8	177, 184, 113
	Cloquintocet-	10 µg	138.7	15.0	0.7	131, 129, 156
	Mexyl EC	33 µg	154.0	16.4	0.8	172, 140, 150
	(A13617AV)	100 µg	181.7	26.6	0.9	151, 199, 195
		333 µg	193.0	24.2	1.0	179, 221, 179
		1000 µg	172.7	12.3	0.9	159, 183, 176
		2500 µg	125.3	23.2	0.6	119 P R, 106 P R, 151 P R
		5000 µg	81.3	1.2	0.4	82 P R, 82 P R, 80 P R
	DMF		194.3	18.7		202, 208, 173
	Untreated Control		202.0	18.1		183, 204, 219

Key to Plate Postfix Codes

P Precipitate
 R Reduced background growth

TABLE 4 Pre-Experiment and Experiment I: 1768500 VV Plate Incorporation (Continued)

Study Name: 1768500
 Experiment: 1768500 VV Plate
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 24.05.2016
 Date Counted: 31.05.2016

With metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts	
WP2 pKM101	Pinoxaden/	3 µg	267.7	23.5	1.1	245, 292, 266	
	Cloquintocet-	10 µg	248.0	32.4	1.0	283, 242, 219	
	Mexyl EC	33 µg	215.0	21.7	0.9	202, 240, 203	
	(A13617AV)	100 µg	273.7	33.1	1.1	239, 277, 305	
		333 µg	238.3	16.6	1.0	256, 236, 223	
		1000 µg	210.3	8.5	0.9	210, 219, 202	
		2500 µg	184.7	19.8	0.8	167 P, 206 P, 181 P	
		5000 µg	204.3	35.8	0.9	163 P, 224 P, 226 P	
	DMF			239.7	8.7		242, 230, 247
	Untreated Control			281.0	9.8		273, 292, 278
WP2 uvrA pKM101	Pinoxaden/	3 µg	398.0	6.9	1.0	394, 406, 394	
	Cloquintocet-	10 µg	406.3	3.8	1.0	402, 409, 408	
	Mexyl EC	33 µg	400.7	18.6	1.0	383, 420, 399	
	(A13617AV)	100 µg	398.0	31.4	1.0	362, 412, 420	
		333 µg	396.0	27.0	1.0	383, 427, 378	
		1000 µg	390.7	48.0	0.9	438, 392, 342	
		2500 µg	352.3	59.0	0.9	420 P, 325 P, 312 P	
		5000 µg	369.7	33.6	0.9	331 P, 386 P, 392 P	
	DMF			412.7	20.5		392, 433, 413
	Untreated Control			444.3	24.1		433, 428, 472
TA 1535	2-AA	2.5 µg	432.0	6.9	34.1	428, 440, 428	
TA 1537	2-AA	2.5 µg	237.0	11.5	20.9	246, 241, 224	
TA 98	2-AA	2.5 µg	4347.7	548.0	118.6	3729, 4542, 4772	
TA 100	2-AA	2.5 µg	5715.7	130.9	29.4	5565, 5802, 5780	
WP2 pKM101	2-AA	10.0 µg	1431.0	37.6	6.0	1472, 1423, 1398	
WP2 uvrA pKM101	2-AA	10.0 µg	2103.0	32.9	5.1	2066, 2129, 2114	

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate

TABLE 5 Experiment II: 1768500 HV2 Pre Incubation

Study Name: 1768500
 Experiment: 1768500 HV2 Pre
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 17.06.2016
 Date Counted: 20.06.2016

Without metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Pinoxaden/	10 µg	10.0	2.6	0.9	7, 11, 12
	Cloquintocet-	33 µg	10.7	0.6	1.0	11, 10, 11
	Mexyl EC	100 µg	11.7	2.5	1.1	14, 12, 9
	(A13617AV)	333 µg	10.3	0.6	1.0	10, 11, 10
		1000 µg	11.0	3.6	1.0	14, 12, 7
		2500 µg	11.0	1.7	1.0	12, 12, 9
		5000 µg	10.7	0.6	1.0	11 P, 11 P, 10 P
	DMF		10.7	0.6		11, 11, 10
	Untreated Control		12.0	2.6		10, 11, 15
TA 1537	Pinoxaden/	33 µg	6.7	3.1	0.7	4, 10, 6
	Cloquintocet-	100 µg	9.7	4.6	1.1	15, 7, 7
	Mexyl EC	333 µg	8.0	1.7	0.9	7, 7, 10
	(A13617AV)	1000 µg	7.3	3.2	0.8	6, 5, 11
		2500 µg	7.7	2.3	0.9	5, 9, 9
		5000 µg	7.3	2.5	0.8	5 P R, 10 P R, 7 P R
		DMF		9.0	3.0	
	Untreated Control		7.3	2.5		7, 10, 5
TA 98	Pinoxaden/	33 µg	24.0	5.3	0.9	22, 30, 20
	Cloquintocet-	100 µg	23.0	3.5	0.9	25, 19, 25
	Mexyl EC	333 µg	22.3	1.2	0.9	23, 21, 23
	(A13617AV)	1000 µg	24.7	2.5	1.0	22, 25, 27
		2500 µg	21.7	2.3	0.8	19, 23, 23
		5000 µg	20.0	1.0	0.8	21 P, 20 P, 19 P
		DMF		25.7	5.5	
	Untreated Control		28.3	9.9		33, 35, 17
TA 100	Pinoxaden/	10 µg	156.7	11.8	1.0	143, 164, 163
	Cloquintocet-	33 µg	178.0	16.5	1.1	179, 161, 194
	Mexyl EC	100 µg	171.7	10.8	1.0	164, 167, 184
	(A13617AV)	333 µg	109.0	24.0	0.7	101, 136, 90
		1000 µg	71.3	15.3	0.4	68, 58, 88
		2500 µg	60.3	4.0	0.4	64, 61, 56
		5000 µg	48.3	8.7	0.3	41 P, 58 P, 46 P
	DMF		164.0	22.1		162, 187, 143
	Untreated Control		200.7	1.2		202, 200, 200

Key to Plate Postfix Codes

P Precipitate
 R Reduced background growth

TABLE 5 Experiment II: 1768500 HV2 Pre Incubation (Continued)

Study Name: 1768500
 Experiment: 1768500 HV2 Pre
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 17.06.2016
 Date Counted: 20.06.2016

Without metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 pKM101	Pinoxaden/	33 µg	208.3	3.8	1.0	211, 210, 204
	Cloquintocet-	100 µg	221.7	6.7	1.0	220, 229, 216
	Mexyl EC	333 µg	210.0	19.3	1.0	227, 214, 189
	(A13617AV)	1000 µg	117.7	16.8	0.5	114, 136, 103
		2500 µg	71.7	3.5	0.3	68, 72, 75
		5000 µg	60.7	8.5	0.3	61 P, 69 P, 52 P
	DMF			217.0	7.9	
Untreated Control			235.0	13.1		241, 244, 220
WP2 uvrA pKM101	Pinoxaden/	33 µg	326.0	7.0	1.0	331, 318, 329
	Cloquintocet-	100 µg	339.3	14.0	1.0	335, 328, 355
	Mexyl EC	333 µg	317.7	9.0	0.9	328, 312, 313
	(A13617AV)	1000 µg	272.3	49.2	0.8	307, 216, 294
		2500 µg	190.0	3.0	0.6	190, 187, 193
		5000 µg	161.3	21.2	0.5	184 P, 158 P, 142 P
	DMF			337.0	15.7	
Untreated Control			315.3	29.4		310, 289, 347
TA 1535	NaN3	10 µg	1179.3	26.9	110.6	1160, 1168, 1210
TA 1537	4-NOPD	50 µg	66.7	13.4	7.4	61, 57, 82
TA 98	4-NOPD	10 µg	364.3	4.9	14.2	370, 362, 361
TA 100	NaN3	10 µg	1891.3	24.0	11.5	1868, 1890, 1916
WP2 pKM101	MMS	2.0 µL	3725.0	197.3	17.2	3542, 3699, 3934
WP2 uvrA pKM101	MMS	2.0 µL	2830.0	106.2	8.4	2780, 2952, 2758
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 6 Experiment II: 1768500 HV2 Pre Incubation

Study Name: 1768500
 Experiment: 1768500 HV2 Pre
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 17.06.2016
 Date Counted: 20.06.2016

With metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts	
TA 1535	Pinoxaden/	10 µg	15.0	1.0	0.9	14, 16, 15	
	Cloquintocet-	33 µg	16.3	7.5	1.0	12, 25, 12	
	Mexyl EC	100 µg	13.7	2.5	0.8	14, 16, 11	
	(A13617AV)	333 µg	11.7	2.9	0.7	15, 10, 10	
		1000 µg	14.3	4.6	0.9	9, 17, 17	
		2500 µg	13.7	3.5	0.8	10, 17, 14	
		5000 µg	13.0	2.6	0.8	16 P, 12 P, 11 P	
	DMF			16.3	5.5		22, 11, 16
	Untreated Control		10.7	1.2		10, 10, 12	
TA 1537	Pinoxaden/	33 µg	14.0	5.3	1.1	12, 10, 20	
	Cloquintocet-	100 µg	16.0	5.6	1.3	21, 17, 10	
	Mexyl EC	333 µg	17.0	6.0	1.3	17, 23, 11	
	(A13617AV)	1000 µg	13.7	1.5	1.1	15, 14, 12	
		2500 µg	18.3	5.5	1.4	12, 21, 22	
		5000 µg	10.3	2.1	0.8	11 P R M, 8 P R M, 12 P R M	
	DMF			12.7	2.9		16, 11, 11
		Untreated Control		12.0	3.0		9, 15, 12
TA 98	Pinoxaden/	33 µg	39.7	1.5	1.0	41, 38, 40	
	Cloquintocet-	100 µg	36.3	9.8	0.9	25, 42, 42	
	Mexyl EC	333 µg	40.0	5.0	1.0	35, 45, 40	
	(A13617AV)	1000 µg	43.0	13.1	1.1	52, 49, 28	
		2500 µg	26.3	7.6	0.7	23, 35, 21	
		5000 µg	29.0	5.2	0.7	26 P, 35 P, 26 P	
	DMF			40.0	8.7		45, 30, 45
		Untreated Control		38.3	2.9		35, 40, 40
TA 100	Pinoxaden/	10 µg	173.3	15.9	0.9	181, 155, 184	
	Cloquintocet-	33 µg	168.0	9.0	0.9	168, 159, 177	
	Mexyl EC	100 µg	175.0	21.7	0.9	187, 188, 150	
	(A13617AV)	333 µg	171.3	15.3	0.9	189, 162, 163	
		1000 µg	90.7	21.1	0.5	115, 77, 80	
		2500 µg	63.3	9.1	0.3	67, 53, 70	
		5000 µg	41.3	14.4	0.2	58 P R, 33 P R, 33 P R	
	DMF			187.0	23.4		173, 174, 214
	Untreated Control		211.3	11.9		206, 203, 225	

Key to Plate Postfix Codes

P Precipitate
 R Reduced background growth
 M Manual count

TABLE 6 Experiment II: 1768500 HV2 Pre Incubation (Continued)

Study Name: 1768500
 Experiment: 1768500 HV2 Pre
 Assay Conditions:

Study Code: Envigo 1768500
 Date Plated: 17.06.2016
 Date Counted: 20.06.2016

With metabolic activation

Strain	Compound	Concentration per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 pKM101	Pinoxaden/	33 µg	241.0	20.0	0.9	236, 263, 224
	Cloquintocet-	100 µg	256.0	8.5	1.0	265, 255, 248
	Mexyl EC	333 µg	223.7	11.9	0.8	237, 220, 214
	(A13617AV)	1000 µg	241.7	21.5	0.9	260, 218, 247
		2500 µg	177.3	9.9	0.7	184, 182, 166
		5000 µg	147.3	5.5	0.6	150 P, 151 P, 141 P
	DMF		266.7	13.8		251, 272, 277
Untreated Control			281.7	13.7		267, 284, 294
WP2 uvrA pKM101	Pinoxaden/	33 µg	403.3	3.2	1.0	402, 407, 401
	Cloquintocet-	100 µg	391.0	16.8	1.0	404, 372, 397
	Mexyl EC	333 µg	382.3	15.0	0.9	378, 399, 370
	(A13617AV)	1000 µg	398.0	9.6	1.0	409, 391, 394
		2500 µg	375.7	6.8	0.9	378, 381, 368
		5000 µg	349.0	22.7	0.9	365 P, 359 P, 323 P
	DMF		407.3	9.5		398, 417, 407
Untreated Control			405.7	14.8		422, 402, 393
TA 1535	2-AA	2.5 µg	399.0	27.2	24.4	424, 403, 370
TA 1537	2-AA	2.5 µg	219.0	25.6	17.3	246, 195, 216
TA 98	2-AA	2.5 µg	5167.0	668.0	129.2	5835, 5167, 4499
TA 100	2-AA	2.5 µg	5103.3	34.8	27.3	5143, 5078, 5089
WP2 pKM101	2-AA	10.0 µg	1200.7	24.0	4.5	1228, 1191, 1183
WP2 uvrA pKM101	2-AA	10.0 µg	2252.7	75.1	5.5	2180, 2330, 2248

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate

TABLE 7 Batch Control of S9

Lot R 140116B		
Treatment	μL S9 / plate	Number of revertants in TA98
Negative Control	0	28
	100	50
10 μg/plate 2-Aminoanthracene	0	32
	100	2982
10 μg/plate Benzo(a)pyrene	0	24
	100	111

APPENDICES SECTION

APPENDIX 1 Historical Control Data

These data represent the laboratory's historical control data from January 2015 until December 2015 representing approx. 450 experiments (WP2 uvrA the historical data are based on approx. 200 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	11	2.15	7	23	12	2.14	7	21
	Untreated control	12	2.97	6	24	12	2.71	7	26
	Positive control	1090	123.80	334	1372	392	62.85	176	549
TA1537	Solvent control	10	1.83	6	18	13	3.27	7	27
	Untreated control	10	2.29	6	20	14	3.72	7	25
	Positive control	83	12.28	55	131	175	44.44	82	327
TA 98	Solvent control	24	3.75	16	36	33	5.55	18	51
	Untreated control	26	4.72	15	43	36	5.83	17	56
	Positive control	344	51.13	211	599	3822	857.83	319	5048
TA 100	Solvent control	155	24.19	84	194	145	31.81	81	204
	Untreated control	174	21.92	90	206	170	23.62	93	212
	Positive control	1956	279.93	658	2528	3606	676.07	722	4940
WP2 pKM 101	Solvent control	207	21.41	171	292	227	24.17	196	332
	Untreated control	223	21.20	169	314	258	22.59	202	334
	Positive control	3757	452.85	2796	4801	1416	568.11	1093	4068
WP2uvrA pKM 101	Solvent control	333	34.45	242	392	374	37.52	276	464
	Untreated control	346	34.13	283	412	386	36.24	296	460
	Positive control	3314	622.93	2163	4571	2112	240.46	1708	2782

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

ENVIGO CRS GmbH
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 studies on biological materials

13. – 16. Juli 2015

Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

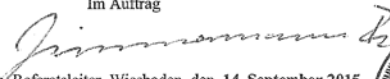
Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

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


Th. Zimmermann, Referatsleiter, Wiesbaden, den 14. September 2015
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)



Hess. Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80 D65189 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 Analysis



GLP Testing Facility WMU Syngenta Crop Protection
Analytical Development & Münchwilen AG
Product Chemistry GS2131 Im Breitenloh 5
4333 Münchwilen, Switzerland

Certificate of Analysis

A13617AV
Pinoxaden/Cloquintocet-mexyl EC (050/012.5)
SMO3K0051

Batch Identification SMO3K0051
Product Code A13617AV
Other Product Code(s) Pinoxaden/Cloquintocet-mexyl EC (050/012.5)

Chemical Analysis
(Active Ingredient Content)

- **Identity of the Active Ingredient(s)*** confirmed
- **Content of Pinoxaden*** 5.23 % w/w corresponding to 50.5 g/l
- **Content of Cloquintocet-mexyl*** 1.35 % w/w corresponding to 13.0 g/l

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

Methodology used for Characterization HPLC, oscillating density meter

Physical Analysis

- **Appearance** amber liquid
- **Density*** 965 kg/m³

Stability:

- **Storage Temperature** < 30°C
- **Recertification Date** End of December 2016

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 Münchwilen AG, Switzerland.

Study number of batch characterization: 126680
Study number(s) of batch recertification:

Authorization: 28-Jan-2014

Dr. Sven Adolph
Analytical Development & Product Chemistry