**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**

## STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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# 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

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# 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

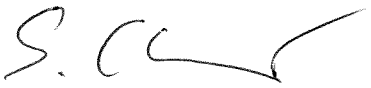
04 August 2016

Date

## PROJECT STAFF SIGNATURE

Study Director

Dr. Steffi Chang



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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) |

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# 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 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 $his^- \rightarrow his^+$ and $trp^- \rightarrow trp^+$ 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 ≥ 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 µg/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 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.: | MKBJ 8702V |
| Purity: | > 99.0 % |
| 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.: | STBD 3302V |
| Purity: | 97.5 % |
| Dissolved in: | DMSO ( purity > 99 %, Fisher Leics LE11 5RG) |
| Concentration: | 2.5 µg/plate (TA1535, TA1537, TA98, TA100),<br>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 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** |
|---|---|---|
| ***Salmonella typhimurium*** | | |
| TA1537 | *his* C 3076; *rfa*$^-$; *uvrB*$^-$ | frame shift mutations |
| TA98 | *his* D 3052; *rfa*$^-$; *uvrB*$^-$; R-factor | " " |
| TA1535 | *his* G 46; *rfa*$^-$; *uvrB*$^-$ | base-pair substitutions |
| TA100 | *his* G 46; *rfa*$^-$; *uvrB*$^-$; R-factor | " " |
| ***Escherichia coli*** | | |
| WP2 *uvrA* pKM101 | *trp* E 56 *uvrA*$^-$; R-factor | base-pair substitutions and others |
| WP2 pKM101 | *trp* E 56; R-factor | " " |

Regular checking of the properties of the *S.typhimurium* and *E. coli* strains regarding the membrane permeability and ampicillin resistance; UV sensitivity, and amino acid requirement as well as normal spontaneous mutation rates is performed by 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 µL ampicillin (25 µg/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: | for *Escherichia coli*: |
|---|---|
| 7.0 g Agar Agar* | 7.0 g Agar Agar* |
| 6.0 g NaCl* | 6.0 g NaCl* |
| 10.5 mg L-Histidine×HCl×$H_2O$* | 10.2 mg Tryptophan* |
| 12.2 mg Biotin* | |

* (MERCK, D-64293 Darmstadt)

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/ß-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 ß-naphthoflavone (Acros Organics, 2440 Geel, Belgium) each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 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:

| | |
|---|---|
| 8 mM | $MgCl_2$ |
| 33 mM | KCl |
| 5 mM | Glucose-6-phosphate |
| 4 mM | NADP |

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames *et al.*(5).

## 3.5 Pre-Experiment for Cytotoxicity

To evaluate the cytotoxicity of the test substance a pre-experiment was performed with all strains. Eight concentrations were tested for cytotoxicity and mutation induction each with three replicate plates. The experimental conditions in this pre-experiment 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. $8x10^8$ 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 (µg/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 *uvrA* 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 (µg/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 *uvrA* 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


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   Mutation Res. 113, 173-215

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6. de Serres F.J. and M.D. Shelby (1979)
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   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 uvrA 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 [P R] | 28 ± 8 [P] | 125 ± 23 [P R] | 185 ± 20 [P] | 352 ± 59 [P] |
| | | 5000 µg | 6 ± 1 [P] | 15 ± 3 [P R] | 29 ± 3 [P R] | 81 ± 1 [P R] | 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

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

Key to Plate Postfix Codes

| | |
|---|---|
| P | Precipitate |
| 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 | Concen-tration (per plate) | Revertant Colony Counts (Mean ±SD) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | TA 1535 | TA 1537 | TA 98 | TA 100 | WP2 pKM101 | WP2 uvrA pKM101 |
| Without Activation | DMF | | 11 ± 1 | 9 ± 3 | 26 ± 6 | 164 ± 22 | 217 ± 8 | 337 ± 16 |
| | Untreated | | 12 ± 3 | 7 ± 3 | 28 ± 10 | 201 ± 1 | 235 ± 13 | 315 ± 29 |
| | Pinoxaden/ | 10 µg | 10 ± 3 | | | 157 ± 12 | | |
| | Cloquintocet- | 33 µg | 11 ± 1 | 7 ± 3 | 24 ± 5 | 178 ± 17 | 208 ± 4 | 326 ± 7 |
| | Mexyl EC | 100 µg | 12 ± 3 | 10 ± 5 | 23 ± 3 | 172 ± 11 | 222 ± 7 | 339 ± 14 |
| | (A13617AV) | 333 µg | 10 ± 1 | 8 ± 2 | 22 ± 1 | 109 ± 24 | 210 ± 19 | 318 ± 9 |
| | | 1000 µg | 11 ± 4 | 7 ± 3 | 25 ± 3 | 71 ± 15 | 118 ± 17 | 272 ± 49 |
| | | 2500 µg | 11 ± 2 | 8 ± 2 | 22 ± 2 | 60 ± 4 | 72 ± 4 | 190 ± 3 |
| | | 5000 µg | 11 ± 1 [P] | 7 ± 3 [P R] | 20 ± 1 [P] | 48 ± 9 [P] | 61 ± 9 [P] | 161 ± 21 [P] |
| | NaN3 | 10 µg | 1179 ± 27 | | | 1891 ± 24 | | |
| | 4-NOPD | 10 µg | | | 364 ± 5 | | | |
| | 4-NOPD | 50 µg | | 67 ± 13 | | | | |
| | MMS | 2.0 µL | | | | | 3725 ± 197 | 2830 ± 106 |
| With Activation | DMF | | 16 ± 6 | 13 ± 3 | 40 ± 9 | 187 ± 23 | 267 ± 14 | 407 ± 10 |
| | Untreated | | 11 ± 1 | 12 ± 3 | 38 ± 3 | 211 ± 12 | 282 ± 14 | 406 ± 15 |
| | Pinoxaden/ | 10 µg | 15 ± 1 | | | 173 ± 16 | | |
| | Cloquintocet- | 33 µg | 16 ± 8 | 14 ± 5 | 40 ± 2 | 168 ± 9 | 241 ± 20 | 403 ± 3 |
| | Mexyl EC | 100 µg | 14 ± 3 | 16 ± 6 | 36 ± 10 | 175 ± 22 | 256 ± 9 | 391 ± 17 |
| | (A13617AV) | 333 µg | 12 ± 3 | 17 ± 6 | 40 ± 5 | 171 ± 15 | 224 ± 12 | 382 ± 15 |
| | | 1000 µg | 14 ± 5 | 14 ± 2 | 43 ± 13 | 91 ± 21 | 242 ± 22 | 398 ± 10 |
| | | 2500 µg | 14 ± 4 | 18 ± 6 | 26 ± 8 | 63 ± 9 | 177 ± 10 | 376 ± 7 |
| | | 5000 µg | 13 ± 3 [P] | 10 ± 2 [P R M] | 29 ± 5 [P] | 41 ± 14 [P R] | 147 ± 6 [P] | 349 ± 23 [P] |
| | 2-AA | 2.5 µg | 399 ± 27 | 219 ± 26 | 5167 ± 668 | 5103 ± 35 | | |
| | 2-AA | 10.0 µg | | | | | 1201 ± 24 | 2253 ± 75 |

Key to Positive Controls

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

Key to Plate Postfix Codes

| | |
|---|---|
| P | Precipitate |
| R | Reduced background growth |
| M | Manual count |

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

Study Name: 1768500 Study Code: Envigo 1768500
Experiment: 1768500 VV Plate Date Plated: 24.05.2016
Assay Conditions: 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/ -** | 3 µg | *13.7* | *7.2* | *1.1* | 22, 10, 9 |
| | **Cloquintocet** | 10 µg | *10.0* | *1.7* | *0.8* | 9, 12, 9 |
| | **Mexyl EC** | 33 µg | *12.0* | *2.6* | *0.9* | 10, 15, 11 |
| | **(A13617AV)** | 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* | | 9, 15, 15 |
| | **Untreated Control** | | *9.7* | *1.2* | | 11, 9, 9 |
| **TA 1537** | **Pinoxaden/ -** | 3 µg | *13.3* | *2.1* | *1.1* | 11, 15, 14 |
| | **Cloquintocet** | 10 µg | *13.0* | *5.2* | *1.0* | 16, 16, 7 |
| | **Mexyl EC** | 33 µg | *10.3* | *1.5* | *0.8* | 12, 10, 9 |
| | **(A13617AV)** | 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* | | 16, 15, 7 |
| | **Untreated Control** | | *11.7* | *4.9* | | 6, 15, 14 |
| **TA 98** | **Pinoxaden/ -** | 3 µg | *27.0* | *5.2* | *1.1* | 30, 30, 21 |
| | **Cloquintocet** | 10 µg | *30.0* | *5.0* | *1.2* | 25, 30, 35 |
| | **Mexyl EC** | 33 µg | *30.0* | *2.0* | *1.2* | 28, 30, 32 |
| | **(A13617AV)** | 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* | | 19, 31, 26 |
| | **Untreated Control** | | *33.3* | *3.5* | | 33, 30, 37 |
| **TA 100** | **Pinoxaden/ -** | 3 µg | *191.3* | *8.1* | *1.2* | 190, 200, 184 |
| | **Cloquintocet** | 10 µg | *188.3* | *5.1* | *1.2* | 194, 187, 184 |
| | **Mexyl EC** | 33 µg | *194.0* | *5.2* | *1.2* | 188, 197, 197 |
| | **(A13617AV)** | 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* | | 162, 137, 190 |
| | **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** | **Pinoxaden/ -** | 3 µg | *214.7* | *19.6* | *0.9* | 220, 231, 193 |
| **pKM101** | **Cloquintocet** | 10 µg | *207.3* | *6.7* | *0.9* | 204, 203, 215 |
| | **Mexyl EC** | 33 µg | *225.7* | *4.6* | *1.0* | 223, 231, 223 |
| | **(A13617AV)** | 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** | **Pinoxaden/ -** | 3 µg | *363.0* | *26.2* | *1.0* | 339, 391, 359 |
| **uvrA** | **Cloquintocet** | 10 µg | *367.7* | *16.3* | *1.0* | 362, 355, 386 |
| **pKM101** | **Mexyl EC** | 33 µg | *356.7* | *0.6* | *1.0* | 357, 357, 356 |
| | **(A13617AV)** | 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 Study Code: Envigo 1768500
Experiment: 1768500 VV Plate Date Plated: 24.05.2016
Assay Conditions: 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/ Cloquintocet-Mexyl EC (A13617AV)** | 3 µg | *267.7* | *23.5* | *1.1* | 245, 292, 266 |
| | | 10 µg | *248.0* | *32.4* | *1.0* | 283, 242, 219 |
| | | 33 µg | *215.0* | *21.7* | *0.9* | 202, 240, 203 |
| | | 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/ Cloquintocet-Mexyl EC (A13617AV)** | 3 µg | *398.0* | *6.9* | *1.0* | 394, 406, 394 |
| | | 10 µg | *406.3* | *3.8* | *1.0* | 402, 409, 408 |
| | | 33 µg | *400.7* | *18.6* | *1.0* | 383, 420, 399 |
| | | 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* | | 6, 12, 9 |
| | **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* | | 22, 23, 32 |
| | **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 Study Code: Envigo 1768500
Experiment: 1768500 HV2 Pre Date Plated: 17.06.2016
Assay Conditions: 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/ Cloquintocet-Mexyl EC (A13617AV)** | 33 µg | *208.3* | *3.8* | *1.0* | 211, 210, 204 |
| | | 100 µg | *221.7* | *6.7* | *1.0* | 220, 229, 216 |
| | | 333 µg | *210.0* | *19.3* | *1.0* | 227, 214, 189 |
| | | 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* | | 226, 214, 211 |
| | **Untreated Control** | | *235.0* | *13.1* | | 241, 244, 220 |
| **WP2 uvrA pKM101** | **Pinoxaden/ Cloquintocet-Mexyl EC (A13617AV)** | 33 µg | *326.0* | *7.0* | *1.0* | 331, 318, 329 |
| | | 100 µg | *339.3* | *14.0* | *1.0* | 335, 328, 355 |
| | | 333 µg | *317.7* | *9.0* | *0.9* | 328, 312, 313 |
| | | 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* | | 326, 355, 330 |
| | **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

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

Key to Plate Postfix Codes

| | |
|---|---|
| P | Precipitate |

# 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/ Cloquintocet-Mexyl EC (A13617AV)** | 33 µg | *241.0* | *20.0* | *0.9* | 236, 263, 224 |
| | | 100 µg | *256.0* | *8.5* | *1.0* | 265, 255, 248 |
| | | 333 µg | *223.7* | *11.9* | *0.8* | 237, 220, 214 |
| | | 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/ Cloquintocet-Mexyl EC (A13617AV)** | 33 µg | *403.3* | *3.2* | *1.0* | 402, 407, 401 |
| | | 100 µg | *391.0* | *16.8* | *1.0* | 404, 372, 397 |
| | | 333 µg | *382.3* | *15.0* | *0.9* | 378, 399, 370 |
| | | 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 | | Key to Plate Postfix Codes | |
|---|---|---|---|
| 2-AA | 2-aminoanthracene | 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

HESSEN

**Gute Laborpraxis/Good Laboratory Practice**

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

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

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

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

**ENVIGO CRS GmbH**
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

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

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

HESSISCHES MINISTERIUM FÜR UMWELT, KLIMASCHUTZ, LANDWIRTSCHAFT UND VERBRAUCHERSCHUTZ

**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**
Analytical Development &
Product Chemistry GS2131

**Syngenta Crop Protection Münchwilen AG**
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/m$^3$** |

**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