**CA5204A**

# CA5204A - Acute Oral Toxicity Study in the Rat (Up and Down Procedure)

**Final Report**

**DATA REQUIREMENT(S):** OECD Test Guideline 425 (2008)
EPA OPPTS 870.1100 (2002)

**AUTHOR(S):** Erika Matting, M.Sc.

**STUDY COMPLETION DATE:** 11 March 2014

**PERFORMING LABORATORY:** CiToxLAB Hungary Ltd.
H-8200 Veszprém, Szabadságpuszta, Hungary

**LABORATORY PROJECT ID:** Report Number: 13/298-001P
Study Number: 13/298-001P
Task Number: TK0103586

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

## STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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# GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study has been performed in accordance with the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 9/2001. (III. 30.) EüM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.).

This study was conducted in accordance with a written Study Plan, authorized by the Sponsor and CiToxLAB Hungary Ltd. Management, and followed applicable Standard Operating Procedures.

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in the course of this study.

Signature: _____ Date: 11 March 2014

Erika Matting, M.Sc.
Study Director

Performing Laboratory: CiToxLAB Hungary Ltd.
H-8200 Veszprém, Szabadságpuszta,
Hungary

# FLAGGING STATEMENT

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# QUALITY ASSURANCE STATEMENT

Study Number: 13/298-001P

Study Title: CA5204A - Acute Oral Toxicity Study in the Rat (Up and Down Procedure)

Test Item: CA5204A

This study has been inspected, and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the study director and to management. The dates of such inspections and of the report audit are given below:

| Date of Inspection | Phase(s) Inspected/Audited | Date of report to | |
|---|---|---|---|
| | | Management | Study Director |
| 08 November 2013 | Study Plan | 08 November 2013 | 08 November 2013 |
| 12 November 2013 | Treatment | 12 November 2013 | 12 November 2013 |
| 13 December 2013 | Draft Report | 13 December 2013 | 13 December 2013 |
| 11 March 2014 | Final Report | 11 March 2014 | 11 March 2014 |

Signature: _____ Date: 11 March 2014

Katalin Böröczki, M.Sc.
QA Inspector

## MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Limited (as Sponsor) and CiToxLAB Hungary Ltd. (as Test Facility) the study titled "CA5204A - Acute Oral Toxicity Study in the Rat (Up and Down Procedure)" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 11 March 2014

Alyson Leyshon, M.Sc.
Managing Director
(formerly Senior VP Business Development Europe and Operations Hungary)

GENERAL INFORMATION

**Contributors**

The following contributed to this report in the capacities indicated:

| **Name** | **Function** |
|---|---|
| Erika Matting, M.Sc. | Study Director |
| Viktória Zelenák, M.Sc. | Assistant Scientist |
| Szabolcs Gáty, M.Sc. | Quality Assurance Unit |
| Katalin Böröczki, M.Sc. | Quality Assurance Unit |
| István Pásztor, DVM | Veterinary Control |
| Peter Maslej, DVM, PhD | Head of Pathology Unit |
| Tamás Mészáros, PhD | Head of Central Dispensary |
| Hannah Hobson, M.Sc. | Syngenta Study Manager |

**Study dates**

| | |
|---|---|
| Experimental Starting Date | 12 November 2013 |
| Experimental Completion Date | 10 December 2013 |
| Receipt of Animals | 06 November 2013 |
| Acclimatisation | At least 6 days |
| Treatment | 12 November 2013 (female no. 4303)<br>14 November 2013 (female no. 4304)<br>19 November 2013 (female no. 4305)<br>21 November 2013 (female no. 4306)<br>26 November 2013 (female no. 4307)<br>28 November 2013 (female no. 4308) |
| Observation | 12 – 26 November 2013 (female no. 4303)<br>14 – 16 November 2013 (female no. 4304)<br>19 November – 03 December 2013 (female no. 4305)<br>21 – 22 November 2013 (female no. 4306)<br>26 November – 10 December 2013 (female no. 4307)<br>28 – 30 November 2013 (female no. 4308) |

**Deviations from the Study Plan**

There was no deviation during the study.

**Performing laboratory test substance reference number**

130211

**Other**

The study documents:
- study plan,
- all raw data,
- sample of the test item,
- original study report and any amendments,
- correspondence

will be archived according to the Hungarian GLP regulations and to applicable SOP's in the archives of CiToxLAB Hungary Ltd. 8200 Veszprém, Szabadságpuszta, Hungary. This is for a period of 15 years.

After the retention time of 15 years has elapsed all the archived materials listed above will be returned to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded.

# TABLE OF CONTENTS

# 1.0 EXECUTIVE SUMMARY

## 1.1 Study Design

An acute oral toxicity (up and down procedure) study was conducted with 6 animals (female RccHan:(WIST) rats). The starting dose was 550 mg/kg bw. Animals were treated with a single oral (gavage) dose of CA5204A at a dose level of 550 or 2000 mg/kg bw followed by a 14 day observation period. The animals were fasted overnight prior to treatment and food was returned approximately 3 hours after dosing.

Surviving animals were observed individually after dosing at 30 minutes, 1, 2, 3, 4 and 6 hours post treatment and once each day for 14 days thereafter. Body weight was measured on Day -1 (prior to removal of food), Day 0 (prior to administration) and weekly thereafter. All surviving animals were examined macroscopically at the end of the observation period. Moreover, the animals found dead were examined macroscopically and body weight was recorded at necropsy.

## 1.2 Results

*550 mg/kg body weight (three animals):*

No mortality was observed at the dose level of 550 mg/kg bw.

Treatment with CA5204A at the dose level of 550 mg/kg bw caused hunched back (3/3), incoordination (3/3), continuous tremors (2/3), piloerection (3/3) and hyperactivity (2/3). Surviving animals were symptom free from 8 days after the treatment.

There were no treatment related body weight changes. Body weights were within the range commonly recorded for this strain and age.

There was no evidence of the macroscopic observations in animals dosed at 550 mg/kg bw and terminated on Day 14.

*2000 mg/kg body weight (three animals):*

Mortality was observed in all three animals on Day 1 or 2 at 2000 mg/kg bw dose level.

Treatment with CA5204A at the dose level of 2000 mg/kg bw caused decreased activity (3/3), hunched back (2/3), prone position (3/3), incoordination (2/3), continuous tremors (3/3), piloerection (3/3), decreased respiratory rate (1/3) and cold to touch (1/3).

## 1.3 Conclusion

Under the conditions of this study, the acute oral median lethal dose ($LD_{50}$) of the test item, CA5204A, was found to be between 550 and 2000 mg/kg bw in female RccHan:(WIST) rats.

Estimated LD50 = 1049 (Based on an assumed sigma of 0.5).
Approximate 95% confidence interval is 550 to 2000.

# 2.0 INTRODUCTION

## 2.1 Purpose

The purpose of the study was to assess the oral toxicity of the test item CA5204A when administered as a single oral gavage dose to female rats at one or more defined dose levels. The results of the study allowed the test item to be ranked according to most classification systems currently in use.

This study was being performed with vertebrate animals as no *in vitro* alternative is available. The study was designed such that the minimum numbers of animals were used.

## 2.2 Guidelines

The study was performed according to the following guidelines:

- OECD Guideline Reference 425 (2008): Acute Oral Toxicity - Up-and-Down Procedure.
- United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.1100 Acute Oral Toxicity EPA 712-C-02-190, December 2002.

## 2.3 Test Facility

This study was performed in an AAALAC-accredited laboratory. The Institutional Animal Care and Use Committee (IACUC) of CiToxLAB Hungary Ltd. reviewed the study plan and authorized the conduct of the study.

# 3.0 MATERIALS AND METHODS

## 3.1 Test Substance

Data as supplied by the Sponsor.

The Certificate of Analysis is presented in Appendix 2.

| | |
|---|---|
| Name: | CA5204A |
| Batch number: | SMU2DP12003 |
| Product code: | CA5204A |
| Purity: | Content of CA5204: 93.2% (w/w) |
| Appearance: | Brown liquid |
| Density: | 1300 g/L |
| Recertification date: | End of September 2015 |
| Storage conditions: | Room temperature (<30$^{o}$C), protected from light and humidity |
| Safety precautions: | Routine safety precautions (lab coat, gloves, goggles, face mask) for unknown materials were applied to assure personnel health and safety. |

### 3.1.1 Identification and receipt

The test item of a suitable chemical purity together with all precautions required in the handling and disposal of the test item were supplied by the Sponsor. The identification of the test item was made in the Central Dispensary Unit of CiToxLAB Hungary Ltd. on the basis of the information provided by Sponsor.

### 3.1.2 Formulation

The test item was administered undiluted.

## 3.2 Experimental Animals

### 3.2.1 Animals

| | |
|---|---|
| Species and strain: | RccHan:(WIST) rats |
| Source: | Harlan Laboratories S.r.l., S.Pietro al Natisone (UD), Zona Industriale Azzida, 57<br>33040, Italy |
| Number of animals: | 6 |
| Sex: | Female rats, nulliparous and non-pregnant. |
| Age when treated: | Young adult rats, 8-11 weeks old. |
| Body weight at dosing: | 166-203 g |
| Hygienic level: | SPF at arrival, standard housing conditions during study |

| | |
|---|---|
| Identification: | The animals were identified by numbers written on the tail with an indelible marker. The cages were marked with individual identity cards with information about study number, sex, cage number, dose group and individual animal number. |
| Justification of strain: | Recognized by international guidelines as a recommended test system |
| Randomization: | Selected by hand at time of delivery. |
| Acclimatization time: | At least 6 days |

### 3.2.2 Husbandry

| | |
|---|---|
| Animal health: | Only healthy animals were used for the test. The health status was certified by the veterinarian. |
| Room number: | 522/3 |
| Housing / Enrichment: | Animals were housed individually in Type II. polypropylene/polycarbonate cages. Rodents are housed with deep wood sawdust bedding to allow digging and other normal rodent activities. |
| Bedding: | Lignocel Bedding for Laboratory Animals was available to animals during the study. |
| Light: | 12 hours daily, from 6.00 a.m. to 6.00 p.m. |
| Temperature: | 19.1 – 22.2 °C |
| Relative humidity: | 35 – 68 % |
| Ventilation: | 15-20 air exchanges/hour |

The temperature and relative humidity were recorded twice daily during the acclimatisation period and throughout the study.

### 3.2.3 Food and feeding

The animals received ssniff® SM R/M "Autoclavable complete diet for rats and mice – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494, Soest, Germany *ad libitum*. The batch number of the lots used in the study were Lot number: 247 9652, expiry date: December 2013 and Lot number: 186 0298, expiry date: May 2014. A detailed description of the contents of the lot used is archived with the raw data at CiToxLAB Hungary Ltd.

### 3.2.4 Water supply and quality control

The animals received tap water, fit for human consumption, *ad libitum*, from the automatic system supplied by the communcal water network. The water was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

The quality control analysis is performed once every three months and microbiological assessment is performed monthly, by Veszprém County Institute of State Public Health and Medical Officer Service (ÁNTSZ, H-8201 Veszprém, József A.u.36., Hungary). Copies of the relevant Certificates of Analysis are retained in the archives at to CiToxLAB Hungary Ltd.

## 3.3 Administration of the Test Item

### 3.3.1 Dosages

Justification of the doses:

An acute oral toxicity (up and down procedure) study was conducted with 6 animals (female RccHan:(WIST) rats). The starting dose was 550 mg/kg bw. Animals were treated with a single oral (gavage) dose of CA5204A undiluted, at dose levels of 550 or 2000 mg/kg bw. The density of the test item is 1300 g/L, therefore the administered dose volume was 0.42 or 1.54 mL/kg bw. The individual dose volumes used at this concentration are shown below.

| Animal Number | Dose [mg/kg body weight] | Volume Dosed [mL] | Mortality |
|---|---|---|---|
| 4303 | 550 | 0.07 | Survived |
| 4304 | 2000 | 0.27 | Died |
| 4305 | 550 | 0.08 | Survived |
| 4306 | 2000 | 0.29 | Died |
| 4307 | 550 | 0.08 | Survived |
| 4308 | 2000 | 0.31 | Died |

Rationale: Oral administration was considered to be an appropriate dose route as it is a possible route of human exposure.

### 3.3.2 Procedure

A single oral (gavage) dose was followed by a 14 day observation period. The night before treatment the animals were fasted. Food, but not water, was withheld overnight. Animals were weighed before dosing and the food was returned 3 hours after the treatment.

Single animals were dosed sequentially following an interval of at least approximately 48 hours. The time intervals between dosing were determined by the onset, duration and severity of toxic signs.

### 3.3.3 Clinical observations

Surviving animals were observed individually after dosing at 30 minutes, then at approximately 1, 2, 3, 4, and 6 hours after dosing and once each day for 14 days thereafter. Individual observations were performed on the skin and fur, eyes and mucous membranes

and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern were assessed.

Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

### 3.3.4 Body weight measurement

The body weights were recorded on Days -1 (prior to removal of food), 0 (prior to administration), 7 and 14. The body weight of animals found dead were recorded at necropsy.

## 3.4 *Post Mortem* Investigations

All surviving animals were euthanised at the end of the observation period by exsanguination under pentobarbital anaesthesia (Release 300 mg/mL (Pentobarbital sodium) A.U.V. inj, Lot No.: 063012, Expiry Date: January 2015, Produced by: Wirtschaftsgenossenschaft deutscher Tierarzte eG, Germany).

All animals were subjected to gross macroscopic evaluation. The cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. All gross pathological changes were recorded for each animal on the post mortem record sheets and the animals were discarded.

### 3.4.1 Material for euthanasia:

| | |
|---|---|
| Name: | Release 300 mg/mL (Pentobarbital sodium) |
| Lot No.: | 063012 |
| Expiry Date: | January 2015 |
| Produced by: | Wirtschaftsgenossenschaft deutscher Tierarzte eG, Germany |

## 3.5 Data Evaluation

Type, severity and duration of clinical observations are described. Body weight and body weight changes are summarised in tabular form. Necropsy findings are described and summarised in tabular form. Data was evaluated using the Acute Oral Toxicity (OECD Test Guidelines 425) Statistical Programme (AOT 425 Stat Pgm).

# 4.0 RESULTS AND DISCUSSION

## 4.1 Mortality

No mortality was observed at dose level of 550 mg/kg bw.
Mortality was observed in all three animals dosed at 2000 mg/kg bw on Day 1 or 2.

## 4.2 Clinical Signs

Treatment with CA5204A at the dose level of 550 mg/kg bw caused hunched back (3/3), incoordination (3/3), continuous tremors (2/3), piloerection (3/3) and hyperactivity (2/3). Surviving animals were symptom free from 8 days after the treatment.

Treatment with CA5204A at the dose level of 2000 mg/kg bw caused decreased activity (3/3), hunched back (2/3), prone position (3/3), incoordination (2/3), continuous tremors (3/3), piloerection (3/3), decreased respiratory rate (1/3) and cold to touch (1/3). Clinical signs are presented in Table 1.

## 4.3 Body Weights

There were no treatment related body weight changes. Body weights were within the range commonly recorded for this strain and age. Individual body weights are presented in Table 2.

## 4.4 Macroscopic Findings

*Surviving animals:*
There was no evidence of the macroscopic observations in animals dosed at 550 mg/kg bw and terminated on Day 14.

*Animals found dead:*
Yellow-brown discoloration of the stomach found in one female (4306) was considered to be potentially related to the administration of the test item.

In addition, dark/red discoloration of the collapsed lungs was seen in all 3 found dead rats. These observations were regarded as agonal or post mortem in nature.

Individual necropsy results are presented in Table 3. The pathology report is presented in Appendix 1.

# 5.0 CONCLUSIONS

Under the conditions of this study, the acute oral median lethal dose ($LD_{50}$) of the test item, CA5204A, was found to be between 550 and 2000 mg/kg bw in female RccHan:(WIST) rats.

Estimated LD50 = 1049 (Based on an assumed sigma of 0.5).
Approximate 95% confidence interval is 550 to 2000.

# TABLES SECTION

## TABLE 1 Individual Findings – Clinical Signs

**DOSE LEVEL: 550 mg/kg bw, Treatment on Day 0** **SEX: FEMALE**

| Cage No. | Animal Number | Observations | Observation days | | | | | | | | | | | | | | Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8-14 | |
| | | | 30' | 1h | 2h | 3h | 4h | 6h | | | | | | | | | |
| 1 | 4303 | Symptom Free | + | + | + | + | + | + | - | - | - | - | - | + | + | + | 15/20 |
| | | Hunched back | - | - | - | - | - | - | + | + | + | + | + | - | - | - | 5/20 |
| | | Incoordination | - | - | - | - | - | - | - | - | - | 1 | 1 | - | - | - | 2/20 |
| | | Tremors (continuous) Whole body | - | - | - | - | - | - | + | + | - | - | - | - | - | - | 2/20 |
| | | Piloerection | - | - | - | - | - | - | + | + | + | + | + | - | - | - | 5/20 |
| 3 | 4305 | Symptom Free | + | + | + | + | + | + | - | - | - | - | - | + | + | + | 15/20 |
| | | Hyperactivity | - | - | - | - | - | - | - | + | - | - | - | - | - | - | 1/20 |
| | | Hunched back | - | - | - | - | - | - | + | + | + | + | + | - | - | - | 5/20 |
| | | Incoordination | - | - | - | - | - | - | - | - | 1 | 1 | 1 | - | - | - | 3/20 |
| | | Piloerection | - | - | - | - | - | - | + | + | - | - | - | - | - | - | 2/20 |
| 5 | 4307 | Symptom Free | + | + | + | + | + | - | - | - | - | - | - | - | - | + | 12/20 |
| | | Hyperactivity | - | - | - | - | - | + | + | - | - | - | - | - | - | - | 2/20 |
| | | Hunched back | - | - | - | - | - | + | + | + | + | + | + | + | + | - | 8/20 |
| | | Incoordination | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | 1/20 |
| | | Tremors (continuous) Whole body | - | - | - | - | - | - | + | + | + | + | + | + | - | - | 6/20 |
| | | Piloerection | - | - | - | - | - | - | + | + | + | + | + | + | + | - | 7/20 |

**Remarks:** + = present - = absent

h = hour (s) ' = minute

# = Found dead

Frequency of observation = number of occurence of observation / total number of observations

Severities: 1 = Slight/Small/Few; 2 = Moderate/Medium; 3 = Marked/Large/Many

# TABLE 1 Individual Findings – Clinical Signs (Continued)

**DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0** **SEX: FEMALE**

| Cage No. | Animal Number | Observations | Observation days | | | | | | | | | | | | | Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8-14 | |
| | | | 30' | 1h | 2h | 3h | 4h | 6h | | | | | | | | | |
| 2 | 4304# | Activity decreased | 1 | 1 | 1 | 2 | 2 | 2 | 2 | | | | | | | | 7/7 |
| | | Hunched back | - | - | + | + | + | + | + | | | | | | | | 5/7 |
| | | Prone position | + | + | - | - | - | - | - | | | | | | | | 2/7 |
| | | Incoordination | - | - | 1 | 1 | 1 | 2 | 2 | | | | | | | | 5/7 |
| | | Tremors (continuous) Whole body | - | - | - | - | + | + | + | | | | | | | | 3/7 |
| | | Piloeerection | - | - | + | + | + | + | + | | | | | | | | 5/7 |
| | | Cold to touch (Whole body) | - | - | - | - | - | - | + | | | | | | | | 1/7 |
| | | Found dead | - | - | - | - | - | - | - | + | | | | | | | - |
| 4 | 4306# | Activity decreased | 1 | 1 | 3 | 3 | 3 | 3 | | | | | | | | | 6/6 |
| | | Prone position | + | + | + | + | + | + | | | | | | | | | 6/6 |
| | | Tremors (continuous) Whole body | + | + | + | + | + | + | | | | | | | | | 6/6 |
| | | Piloeerection | + | + | + | + | + | + | | | | | | | | | 6/6 |
| | | Found dead | - | - | - | - | - | - | + | | | | | | | | - |
| 6 | 4308# | Activity decreased | - | 1 | 2 | 2 | 3 | 3 | 3 | | | | | | | | 6/7 |
| | | Hunched back | + | + | + | - | - | - | - | | | | | | | | 3/7 |
| | | Prone position | - | - | - | + | + | + | + | | | | | | | | 4/7 |
| | | Incoordination | - | 1 | 1 | - | - | - | - | | | | | | | | 2/7 |
| | | Tremors (continuous) Whole body | + | + | + | - | - | - | - | | | | | | | | 3/7 |
| | | Piloeerection | - | + | + | + | + | + | + | | | | | | | | 6/7 |
| | | Respiratory rate decreased | - | - | - | - | - | 2 | 2 | | | | | | | | 2/7 |
| | | Found dead | - | - | - | - | - | - | - | + | | | | | | | - |

**Remarks:** + = present - = absent

h = hour (s) ' = minute

# = Found dead

Frequency of observation = number of occurence of observation / total number of observations

Severities: 1 = Slight/Small/Few; 2 = Moderate/Medium; 3 = Marked/Large/Many

## TABLE 2 Body Weight and Body Weight Gain

**DOSE LEVEL: 550 mg/kg bw, Treatment on Day 0** **SEX: FEMALE**

| Cage No. | Animal Number | Body weight (g) Days | | | | Body Weight Gain (g) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | -1 | 0 | 7 | 14 | -1-0 | 0-7 | 7- 14 | -1 - 14 |
| 1 | 4303 | 182 | 166 | 171 | 197 | -16 | 5 | 26 | 15 |
| 3 | 4305 | 195 | 187 | 194 | 211 | -8 | 7 | 17 | 16 |
| 5 | 4307 | 216 | 195 | 200 | 226 | -21 | 5 | 26 | 10 |
| **Mean:** | | 197.7 | 182.7 | 188.3 | 211.3 | -15.0 | 5.7 | 23.0 | 13.7 |
| **Standard deviation:** | | 17.2 | 15.0 | 15.3 | 14.5 | 6.6 | 1.2 | 5.2 | 3.2 |

**DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0** **SEX: FEMALE**

| Cage No. | Animal Number | Body weight (g) Days | | | | Body Weight Gain (g) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | -1 | 0 | 7 | 14 | -1-0 | 0-7 | 7- 14 | -1 - 14 |
| 2 | 4304# | 190 | 176 | - | - | -14 | - | - | - |
| 4 | 4306# | 199 | 188 | - | - | -11 | - | - | - |
| 6 | 4308# | 213 | 203 | - | - | -10 | - | - | - |
| **Mean:** | | 200.7 | 189.0 | - | - | -11.7 | - | - | - |
| **Standard deviation:** | | 11.6 | 13.5 | - | - | 2.1 | - | - | - |

- = No data

# = Found dead

## TABLE 3 Macroscopic Findings

**DOSE LEVEL: 550 mg/kg bw, Treatment on Day 0** **SEX: FEMALE**

| Cage No. | Animal Number | Necropsy Day | External Observations | Internal Observations | Organ/Tissue |
|---|---|---|---|---|---|
| 1 | 4303 | Day 14 | No external observations recorded | No internal observations recorded | Not applicable |
| 3 | 4305 | Day 14 | No external observations recorded | No internal observations recorded | Not applicable |
| 5 | 4307 | Day 14 | No external observations recorded | No internal observations recorded | Not applicable |

**DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0** **SEX: FEMALE**

| Cage No. | Animal Number | Necropsy Day | External Observations | Internal Observations | Organ/Tissue |
|---|---|---|---|---|---|
| 2 | 4304# | Day 2 | No external observations recorded | Collapsed | Lungs |
| | | | | Dark discoloration, red, diffuse, all lobes | |
| 4 | 4306# | Day 1 | No external observations recorded | Collapsed | Lungs |
| | | | | Dark discoloration, red, diffuse, all lobes | |
| | | | | Discoloration, yellow-brown diffuse, glandular and non-glandular mucosa | Stomach |
| 6 | 4308# | Day 2 | No external observations recorded | Collapsed | Lungs |
| | | | | Dark discoloration, red, diffuse, all lobes | |

# = Found dead

# APPENDICES SECTION

# APPENDIX 1 Pathology Report

**PATHOLOGY REPORT**

**INTRODUCTION**

The objective of the study was to assess the acute oral toxicity of CA5204A when administered in a single dose to rats at a dose level 550 or 2000 mg/kg bw. The results of the study allows the calculation of the estimated oral $LD_{50}$ of the test item and permits the test item to be ranked according to most classification systems currently in use.

**RESULTS AND DISCUSSION**

Surviving animals were euthanized upon completion of the observation period on Day 14. Rats were anesthetized with pentobarbital, followed by exsanguination. Gross pathology consisted of an external examination, including identification of all clinically-recorded lesions, as well as a detailed internal examination. Histopathological examination was not performed.

**MORTALITY**

Three females were found dead on Day 1 or 2.

**FOUND DEAD**

**Macroscopic Findings**

Yellow-brown discoloration of the stomach found in one female (4306) was considered to be potentially related to the administration of the test item.

In addition, dark/red discoloration of the collapsed lungs was seen in all 3 found dead rats. These observations were regarded as agonal or post mortem in nature.

**TERMINAL (DAY 14)**

**Macroscopic Findings**

There was no evidence of the macroscopic observations in animals dosed at 550 mg/kg bw and terminated on Day 14.

**CONCLUSION**

A single oral gavage of CA5204A to the RccHan: (WIST) female rat dosed at 2000 mg/kg bw led to the death of three females. Yellow-brown discoloration of the stomach macroscopically found in one female was considered to be potentially related to the administration of the test item.

In surviving animals received 550 mg/kg bw and terminated on Day 14, no gross observations were noted.

10 March 2014

Peter Maslej, D.V.M., Ph.D. Date
Head, Pathology Department

## APPENDIX 2 Certificate of Analysis

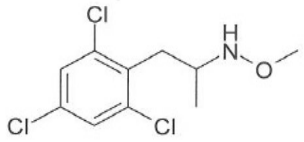
syngenta

GLP Testing Facility WMU
Analytical Development &
Product Chemistry GS2131

Syngenta Crop Protection
Münchwilen AG
Im Breitenloh 5
4333 Münchwilen, Switzerland

### Certificate of Analysis

**CA5204A**



**SMU2DP12003**
**Purity 93.2 %**

| | |
|---|---|
| **Batch Identification** | **SMU2DP12003** |
| **Product Code** | **CA5204A** |
| Other Product Code(s) | CA5204 tech. |
| ISO Common Name | --- |
| CA Reg. No. | --- |
| CA Index Name | --- |
| IUPAC Name | N-methoxy-1-(2,4,6-trichlorophenyl)propan-2-amine |
| Molecular formula | $C_{10}H_{12}Cl_3NO$ |
| Molecular mass | 268.6 |
| **Chemical Analysis** | |
| – **Identity*** | **confirmed** |
| – **Content of CA5204*** | **93.2 % w/w** |
| Methodology used for Characterization | HPLC, $^1$H-NMR (qualitative, quantitative) |
| **Physical Analysis** | |
| – **Appearance*** | **brown liquid** |

**Stability:**

| | |
|---|---|
| – **Storage Temperature** | **< 30°C** |
| – **Recertification Date** | **End of September 2015** |

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: 126261
Study number(s) of batch recertification: ---

Authorization: 04-October-2013

Dr. Christian Mink
Analytical Development & Product Chemistry

## APPENDIX 3 GLP Certificate

GYEMSZI
National Institute for Quality- and Organizational Development in Healthcare and Medicines

National Institute of Pharmacy

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H-1372 Budapest, PO Box 450.
Tel.: +36 1 886 9300
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Web: www.ogyi.hu

**Ref. no: OGYI/38593-5/2012**
**Admin.**: Urbin Magdolna Zita
**Date:** 3 December, 2012

**GOOD LABORATORY PRACTICE (GLP)**
**CERTIFICATE**

It is hereby certified that the test facility

**CiToxLAB Hungary Ltd.**

**H-8200 Veszprém, Szabadságpuszta**

is able to carry out

**physico-chemical testing, toxicity studies, mutagenicity studies, environmental toxicity studies on aquatic or terrestrial organisms, studies on behaviour in water, soil and air; bio-accumulation, reproduction toxicology, inhalation toxicology, analytical chemistry and contract archiving**

in compliance with the Principles of GLP (Good Laboratory Practice) and also complies with the corresponding OECD/European Community requirements.

Date of the inspection: **8-11. October 2012.**

Dr. Kőszeginé Dr. Szalai Hilda
Deputy Director-General