SYN546039

SYN546039 - Acute Oral Toxicity Study in Rats: Up-and-Down-Procedure

Final Report

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

AUTHOR(S): Dr. Maximilian Sieber

STUDY COMPLETION DATE: 29-Sep-2011

PERFORMING LABORATORY: Harlan Laboratories Ltd.
Zelgliweg 1
4452 Itingen, Switzerland

LABORATORY PROJECT ID: Report Number: D35364
Study Number: D35364
Task Number: TK0002682

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

NOTE: This report contains colour page(s).

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

This page is intentionally left blank.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

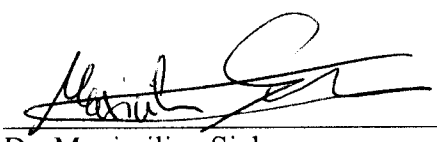
This study has been performed in compliance with the:

Swiss Ordinance relating to Good Laboratory Practice adopted May 18th, 2005 [SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted on November 26th, 1997, by decision of the OECD Council [C (97)186/Final].

These principles 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).

The stability of the test item dilutions under the test conditions is unknown. The formulation trials were performed before the study initiation date. Therefore, they are excluded from this statement.

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



Dr. Maximilian Sieber
Study director

29/09/2011
Date

Performing laboratory: Harlan Laboratories Ltd.
Zelgliweg 1
4452 Itingen, Switzerland

FLAGGING STATEMENT

This page is intentionally left blank.

QUALITY ASSURANCE STATEMENT

Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland

Harlan Laboratories study:	D35364
Syngenta task number:	TK0002682
Test item:	SYN546039
Study director:	Dr. Maximilian Sieber
Study title:	SYN546039 – Acute Oral Toxicity Study in Rats: Up-and-Down-Procedure

The general facilities and activities are inspected at least once a year and the results are reported to the responsible person and the management.

Study procedures were periodically inspected. The study plan and this report were audited by the quality assurance. The dates are given below.

Dates and types of QA inspections		**Dates of reports to the study director and test facility management**
01-Jul-2011	Study plan	01-Jul-2011
15-Jul-2011	Process based (clinical signs)	15-Jul-2011
06-Sep-2011	Report	06-Sep-2011

This statement also confirms that this final report reflects the raw data.

Quality assurance: for Dr. Zeljka Bratoljic-Melkay

Date: 27-Jun-2011

GENERAL INFORMATION

Study title:	SYN546039 – Acute Oral Toxicity Study in Rats: Up-and-Down-Procedure
Sponsor:	Syngenta Ltd Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY, UK
Syngenta study manager:	Claire Elliott
Test facility:	Harlan Laboratories Ltd. Zelgliweg 1 4452 itingen, Switzerland
QA:	Harlan Laboratories Ltd. Quality Assurance GLP Zelgliweg 1 4452 Itingen, Switzerland QAContact_crs.ch@harlan.com

Contributors

The following contributed to this report in the capacities indicated:

Name	**Function**
Dr. Maximilian Sieber	Study director
Dr. Christina Simon	Deputy study director
Caroline Weng	Planning coordinator
Roland Sacher	Laboratory coordinator
Claire Elliott	Syngenta study manager

Study dates

Experimental starting date:	07-Jul-2011
Delivery of animals:	07-Jul-2011
Acclimatization:	07-Jul-2011 to 11-Jul-2011 (animal no. 1) 07-Jul-2011 to 13-Jul-2011 (animal no. 2) 07-Jul-2011 to 17-Jul-2011 (animal no. 3) 07-Jul-2011 to 19-Jul-2011 (animal no. 4) 07-Jul-2011 to 21-Jul-2011 (animal no. 5)

Treatment:	12-Jul-2011 (animal no. 1) 14-Jul-2011 (animal no. 2) 18-Jul-2011 (animal no. 3) 20-Jul-2011 (animal no. 4) 22-Jul-2011 (animal no. 5)
Observations after treatment:	12-Jul-2011 to 26-Jul-2011 (animal no. 1) 14-Jul-2011 to 28-Jul-2011 (animal no. 2) 18-Jul-2011 to 01-Aug-2011 (animal no. 3) 20-Jul-2011 to 03-Aug-2011 (animal no. 4) 22-Jul-2011 to 05-Aug-2011 (animal no. 5)
Experimental completion date:	05-Aug-2011

Animal welfare

This study was performed in an AAALAC-accredited laboratory in accordance with the Swiss Animal Protection Law under license no. 254.

Other

Harlan Laboratories Ltd. (4452 Itingen, Switzerland) will retain the study plan (general study plan / study-specific supplement), raw data, a sample of the test item and the original final report of the present study for at least ten years. No data will be discarded without the Sponsor's written consent.

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

1.1 Study Design

Five female RccHan: WIST(SPF) rats were treated with SYN546039 by oral gavage at the dose levels of 175, 550 and 2000 mg/kg body weight. The test item was applied formulated in 0.5% aqueous carboxymethyl cellulose (CMC) at concentrations of 17.5, 55 and 200 mg/mL and at a dose volume of 10 mL/kg.

The animals were examined daily during acclimatization, assessing for mortality and clinical signs. All animals were examined for clinical signs before treatment, once within the first 30 minutes and approximately 1, 2, 3 and 5 hours after treatment on test day 1 and once daily during test days 2 to 15. Mortality was assessed before treatment, once within the first 30 minutes and approximately 1, 2, 3 and 5 hours after treatment on test day 1 (with the clinical signs) and twice daily during test days 2 to 15. Body weights were recorded on the last day of acclimatization (prior to removal of food), on test day 1 (prior to treatment) and on test days 8 and 15. All animals were examined macroscopically after being sacrificed at the end of the study.

1.2 Results

No deaths occurred during the course of the study.

No clinical signs were recorded in the animals treated with 175 and 550 mg/kg body weight. Ruffled fur was observed in two of the three animals treated with 2000 mg/kg body weight after treatment on test day 1 and lasted in one of these animals up to test day 3. No clinical signs were observed from test day 4 until the end of the study.

The body weights were within the range commonly recorded for this strain and age.

No macroscopic findings were recorded at necropsy.

1.3 Conclusion

The median lethal dose of SYN546039 after single oral administration to female rats, observed over a period of 14 days, is:

LD_{50} (female rat): greater than 2000 mg/kg body weight

2.0 INTRODUCTION

2.1 Purpose

The purpose of this study was to investigate the acute oral toxicity of the test item using the Modified Up-and-Down Procedure (ASTM, 1987).

2.2 Guidelines

The study was conducted 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-03-190, December 2002.

3.0 MATERIALS AND METHODS

3.1 Test Item

The following information was provided by the Sponsor (for certificate of analysis see Appendix 1):

Identification:	SYN546039
Other product code(s):	CSCD695908
Batch No.:	MES 139/4
Purity:	98% w/w
Description:	Beige solid
Expiry Date (Retest Date):	End of June 2013 (as given by the Sponsor) 30-Jun-2013 (as handled by Harlan Laboratories Ltd.)
Storage Conditions:	< 10°C (as given by the Sponsor) Refrigerator (5 ± 3 °C, provided by Harlan Laboratories Ltd.)

3.2 Vehicle

A solubility test was performed before the study initiation date. A 20% (weight:weight) mixture of the test item with purified water formed 2 phases. A 20% (w/w) mixture of the test item with 0.5% CMC formed a beige suspension suitable for oral gavage application.

The following was provided by Harlan Laboratories Ltd.:

Identification:	carboxymethyl cellulose (CMC)
Description:	White powder
Batch Number:	0001414644
Source:	FLUKA Chemie AG, 9471 Buchs, Switzerland
Stability of the Vehicle:	Stable under storage conditions
Expiry Date:	August 2014
Storage Conditions:	At room temperature (range of 20 ± 5 °C), light protected.

A 0.5% aqueous CMC solution was prepared with purified water. This solution was used for the dose formulations and was stored in the refrigerator for a maximum of 1 week.

Purified water was prepared at Harlan Laboratories Ltd. (deionised water which was processed and treated by the PURELAB Option-R unit, linking four purification technologies: reverse osmosis, adsorption, ion-exchange and photo oxidation).

3.3 Experimental Design

The animals received a single dose of the test item by oral gavage administration after being fasted for approximately 17 to 20 hours, but with free access to water. Food was presented approximately 3 hours after dosing.

Dose formulations were prepared shortly before each treatment. The test item was weighed into a tared glass beaker on a suitable precision balance and the vehicle added (w/v). The dose formulation was homogenized using a spatula and an Ultraturrax.

Before administration, homogeneity of the dose formulation was maintained using a magnetic stirrer.

Dosing started in one female animal at a dose level of 175 mg/kg. The application volume was 10 mL/kg body weight.

Dose Levels

Animal no.	Dose [mg/kg body weight]	Volume [mL/kg body weight]
1	175	10
2	550	10
3	2000	10
4	2000	10
5	2000	10

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

The animals were examined daily during acclimatization and mortality and clinical signs were assessed. All animals were examined for clinical signs before treatment, once within the first 30 minutes and approximately 1, 2, 3 and 5 hours after treatment on test day 1 and once daily during test days 2 to 15. Mortality was assessed before treatment, once within the first 30 minutes and approximately 1, 2, 3 and 5 hours after treatment on test day 1 (with the clinical signs) and twice daily during test days 2 to 15. Body weights were recorded on the last day of acclimatization (prior to removal of food), on test day 1 (prior to treatment) and on test days 8 and 15. All animals were examined macroscopically after being sacrificed at the end of the study.

3.4 Animals

Animal species and strain:	Rat, RccHan: WIST(SPF)
Rationale:	Recognized by international guidelines as a recommended test system.
Breeder / supplier:	Harlan Laboratories B.V. Kreuzelweg 53 5961 Horst, The Netherlands
Number of animals per group:	One female
Total number of animals:	5 females
Age (at treatment):	10 - 11 weeks
Body weights (at treatment):	177.8 -198.2 g

Identification:	Unique cage number and corresponding colour-coded spots on the tail. The animals were marked at acclimatization start.
Randomization:	Randomly selected by hand at time of delivery. No computer generated randomization program.
Acclimatization:	Five to fifteen days under laboratory conditions, after health examination. Only animals without any visible signs of illness were used for the study.

3.5 Husbandry

Room number:	E21 / Harlan Laboratories Ltd., Itingen
Conditions:	Standard laboratory conditions. Air-conditioned with 10 - 15 air changes per hour, continuously monitored environmental conditions (temp. range: 22 ± 3 °C; relative humidity range: 30 - 70%). Values outside of these ranges occasionally occurred, usually following room cleaning, and are considered not to have any influence on the study. Therefore, these data are not reported but are retained at Harlan Laboratories Ltd. There was 12-hour fluorescent light/12-hour dark cycle with music during the light period.
Accommodation:	Individually in Makrolon type-3 cages with standard softwood bedding (‘Lignocel’ J. Rettenmaier&Söhne GmbH&CoKG, 73494 Rosenberg, Germany, imported by Provimi Kliba AG, 4303 Kaiseraugst, Switzerland) including paper enrichment (batch no. 75, Enviro-dri from Lillico Biotechnology, Surrey, UK) during treatment and observation.
Diet:	Pelleted Teklad rat-mouse diet 2914C (batch no. 12/11, provided by Provimi Kliba AG, 4303 Kaiseraugst, Switzerland) *ad libitum*, except for the overnight fasting period. Results of analyses for contaminants were archived at Harlan Laboratories Ltd.

Water:	Community tap water from Itingen *ad libitum* in water bottles. Results of bacteriological, chemical and contaminant analyses were archived at Harlan Laboratories Ltd.

3.6 *Post Mortem* Investigations

All animals were sacrificed at the end of the observation period by carbon dioxide asphyxiation and discarded after macroscopic abnormalities were recorded. An external examination and opening of the abdominal and thoracic cavities for examinations of major organs were performed. The appearance of any macroscopic abnormalities was recorded. No organs or tissues were retained.

3.7 Data Compilation

Viability/mortality was recorded on data sheets.

Body weights were recorded on-line with the ToxControl computer system.

Clinical signs and macroscopic findings were compiled into the ToxControl computer system during recording.

The ToxControl computer system has been licensed for Harlan Laboratories Ltd. and validated with respect to data collection, storage and retrievability.

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

(See mortality table)

No deaths occurred during the course of the study.

4.2 Clinical Signs

(See clinical signs table)

No clinical signs were recorded in the animals treated with 175 and 550 mg/kg body weight (animal nos. 1 and no 2, respectively). Ruffled fur was observed in two of the three animals treated with 2000 mg/kg body weight after treatment on test day 1 (animal nos. 4 and no. 5) and lasted in one of these animals (animal no. 5) up to test day 3. No clinical signs were observed from test day 4 until the end of the study.

4.3 Body Weights

(See body weights table)

The body weights were within the range commonly recorded for this strain and age.

4.4 Macroscopic Findings

(See macroscopic findings table)

No macroscopic findings were recorded at necropsy.

5.0 CONCLUSIONS

The median lethal dose of SYN546039 after single oral administration to female rats, observed over a period of 14 days, is:

LD_{50} (female rat): greater than 2000 mg/kg body weight

6.0 REFERENCES

Listed literature references are available upon request.

1. ASTM (1987). Standard Test Method for Estimating Acute Oral Toxicity in Rats. American Society for Testing and Materials, Philadelphia, PA, E 1163 - 1187.

2. Acute Oral Toxicity (OECD Test Guideline 425) Statistical Programme (AOT 425 Stat Pgm). Version: 1.0, 2001 (http://www.epa.gov/oppfead1/harmonization)).

TABLES SECTION

Mortality

Animal no.	Dose [mg/kg]	Day of mortality [test day]	Survival / Death [O/X]
1	175	15	O
2	550	15	O
3	2000	15	O
4	2000	15	O
5	2000	15	O

Clinical Signs

Animal no.	Dose [mg/kg]	Clinical signs (maximum grade)*	Test day																		
			1°						2	3	4	5	6	7	8	9	10	11	12	13	14	15
			0	0.5	1	2	3	5														
1	175	No abnormality recorded.																				
2	550	No abnormality recorded.																				
3	2000	No abnormality recorded.																				
4	2000	Ruffled fur (3)	.	.	.	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	2000	Ruffled fur (3)	.	.	1	1	2	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.

* The following grading system was used: grade 1 = slight (present for maximum grade 1), grade 2 = moderate, grade 3 = marked

° On treatment day, animals were observed prior to test item administration (0 hours), within the first 30 minutes after test item administration, as well as 1, 2, 3 and 5 hours after test item administration.

No abnormalities were recorded during the acclimatization.

Body Weights

Animal no.	Dose [mg/kg]	Body weight [g]				
		Last day of acclimatization	Test day 1	Test day 8	Test day 15	Gain (test days 1-15)
1	175	187.4	177.8	202.1	220.4	42.6
2	550	196.3	184.4	208.2	218.7	34.3
3	2000	207.1	198.2	216.8	236.1	37.9
4	2000	195.2	182.0	205.7	207.7	25.7
5	2000	200	188.2	201.2	210.8	22.6

Macroscopic Findings

Animal no.	Dose [mg/kg]	Day of mortality [test day]	Macroscopic findings
1	175	15	No findings noted.
2	550	15	No findings noted.
3	2000	15	No findings noted.
4	2000	15	No findings noted.
5	2000	15	No findings noted.

APPENDICES SECTION

APPENDIX 1 Certificate of Analysis

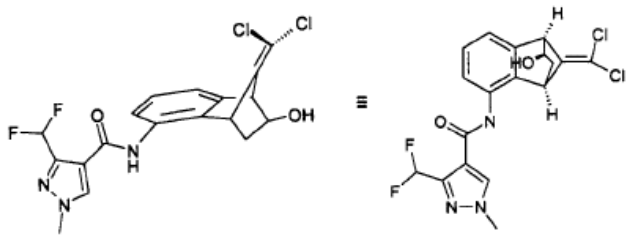
syngenta

GLP Testing Facility WMU
Analytical Development &
Product Chemistry GS2131

Syngenta Crop Protection
Münchwilen AG
Breitenloh 5
CH-4333 Münchwilen

Certificate of Analysis

SYN546039



MES 139/4 - Purity 98 % w/w

Batch Identification	**MES 139/4**
Product Code	**SYN546039**
Parent	SYN545192
Other Product Code(s)	CSCD695908
ISO Common Name	---
CA Reg. No.	---
CA Index Name	---
IUPAC Name	Racemic mixture of 3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid ((1S,2S,4R)-9-dichloromethylene-2-hydroxy-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide and 3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid ((1R,2R,4S)-9-dichloromethylene-2-hydroxy-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide
Molecular formula	$C_{18}H_{15}Cl_2F_2N_3O_2$
Molecular mass	414.2
Chemical Analysis	
– **Identity ***	**confirmed**
– **Content of SYN546039***	**98 % w/w** (estimated error: ± 2 %)
Methodology used for Characterization / Recertification	HPLC, NMR, Titration (Karl Fischer)
Physical Analysis	
– **Appearance ***	**beige solid**
Stability:	
– **Storage Temperature**	**< 10°C**
– **Recertification Date**	**End of June 2013**

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 Muenchwilen AG.

Study number of batch characterization: 123047
Study number(s) of batch recertification:
Authorization:

S. De Benedictis
Analytical Development & Product Chemistry

APPENDIX 2 GLP Certificate

The Swiss GLP Monitoring Authorities

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Home Affairs DHA
Federal Office of Public Health FOPH

Federal Department of the Environment, Transport, Energy and Communications DETEC
Federal Office for the Environment FOEN

swissmedic
Swiss Agency for Therapeutic Products

Statement of GLP Compliance

According to Article 14 paragraph 3 Ordinance on Good Laboratory Practice [OGLP, SR 813.112.1]

The notification authority for chemicals confirms that the following test facility was inspected with respect to the compliance with the Swiss Ordinance on Good Laboratory Practice, adopted on 18th May 2005 [OGLP, SR 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted on 26th November 1997 by decision of the OECD Council [C(97)186/Final].

Unequivocal name and address of the test facility:	Areas of expertise according to article 3 paragraph 1 letter d OGLP:
Harlan Laboratories Ltd Toxicology and Environmental Safety & Metabolism Zelgliweg 1 4452 Itingen, Switzerland (included Test site in 4414 Füllinsdorf, Switzerland)	1. toxicity studies, 3. environmental toxicity studies on aquatic and terrestrial organisms, 4. studies on behaviour in water, soil and air; bioaccumulation, 5. residue studies, 6. studies on effect on mesocosms and natural ecosystems, 7. physical-chemical testing, 8. analytic and clinical chemistry testing, 9. other studies (Safety Pharmacology, Animal metabolism).

Inspection authority: Federal Office for the Environment (FOEN), Federal Office of Public Health (FOPH) and Swiss Agency for Therapeutic Products (Swissmedic)

Date of inspection: 22 to 26 March and 12 to 16 April 2010

Date of decision: 19 November 2010

Based on the above mentioned decision it can be confirmed that the above mentioned test facility is able to conduct studies according to the aforementioned areas of expertise in compliance with the principles of GLP. The above mentioned test facility is listed in the register and GLP list according to the Article 14 OGLP and is inspected on a regular basis according to Article 6 paragraph 2 OGLP.

Swiss Federal Office of Public Health
Consumer protection directorate
Notification authority for chemicals
CH-3003 Bern

Dag Kappel

FEDERAL OFFICE OF PUBLIC HEALTH

Bern, 11 January 2011, The Head, Dr. Dag Kappes.

The notification authority for chemicals is the coordination and decision authority for the good laboratory practice (GLP) for the FOEN, the FOPH and Swissmedic.
Swiss Federal Office of Public Health, Consumer protection directorate, Notification authority for chemicals, CH-3003 Bern.
www.glp.admin.ch, Phone: +41 (0)31 322 73 05, Fax: +41 (0)31 323 54 86