Prosulfocarb/Pyroxsulam/Cloquintocet-mexyl

Prosulfocarb/Pyroxsulam/Cloquintocet-mexyl SE (A18664A) - Acute Oral Toxicity Study in the Rat (Up and Down Procedure)

Final Report

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

AUTHOR(S): Dr. M. Mallaun

STUDY COMPLETION DATE: 08-Apr-2011

PERFORMING LABORATORY: Harlan Laboratories Ltd.
Wölferstrasse 1
4414 Füllinsdorf, Switzerland

LABORATORY PROJECT ID: Report Number: D13033
Study Number: D13033
Task Number: TK0047754

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

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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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).

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

Dr. M. Mallaun
Study director

08-Apr-2011
Date

Performing laboratory: Harlan Laboratories Ltd.
Wölferstrasse 4
4414 Füllinsdorf, Switzerland

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

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

Harlan Laboratories study:	D13033
Syngenta task number:	TK0047754
Test item:	Prosulfocarb/Pyroxsulam/Cloquintocet-mexyl SE (A18664A)
Study director:	Dr. M. Mallaun
Study title:	Prosulfocarb/Pyroxsulam/Cloquintocet-mexyl SE (A18664A)– Acute Oral Toxicity Study in the Rat (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**
17-Jan-2011	Study plan	17-Jan-2011
13-Jan-2011	Process based (test item, dose preparation)	13-Jan-2011
23/24-Mar-2011	Report	24-Mar-2011

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

Quality assurance: for A. Keiner

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Date: 08-Apr-2011

GENERAL INFORMATION

Study title:	Prosulfocarb/Pyroxsulam/Cloquintocet-mexyl SE (A18664A)– Acute Oral Toxicity Study in the Rat (Up and Down Procedure)
Sponsor:	Syngenta Ltd Jealott's Hill International Research Centre Bracknell, Berkshire, RG42 6EY, UK
Syngenta study manager:	C. Elliott
Test facility:	Harlan Laboratories Ltd. Wölferstrasse 4 4414 Füllinsdorf, Switzerland
QA:	Harlan Laboratories Ltd. Quality Assurance GLP Zelgliweg 1 4452 Itingen, Switzerland

Contributors

The following contributed to this report in the capacities indicated:

Name	**Function**
Dr. M. Mallaun	Study director
G. Arcelin	Deputy study director
C. Weng	Laboratory coordinator
T. Fink	Head of quality assurance
C. Elliott	Syngenta study manager

Study dates

Experimental starting date:	18-Jan-2011
Delivery of animals:	18-Jan-2011 (nos. 1-2) 25-Jan-2011 (nos. 3-4) 03-Feb-2011 (no. 5)

Acclimatization:	18-Jan-2011 to 24-Jan-2011 (no. 1)
	18-Jan-2011 to 26-Jan-2011 (no. 2)
	25-Jan-2011 to 31-Jan-2011 (no. 3)
	25-Jan-2011 to 02-Feb-2011 (no. 4)
	03-Feb-2011 to 07-Feb-2011 (no. 5)
Treatment:	25-Jan-2011 (no. 1)
	27-Jan-2011 (no. 2)
	01-Feb-2011 (no. 3)
	03-Feb-2011 (no. 4)
	08-Feb-2011 (no. 5)
Observations after treatment:	25-Jan-2011 to 08-Feb-2011 (no. 1)
	27-Jan-2011 to 10-Feb-2011 (no. 2)
	01-Feb-2011 to 15-Feb-2011 (no. 3)
	03-Feb-2011 to 17-Feb-2011 (no. 4)
	08-Feb-2011 to 22-Feb-2011 (no. 5)
Experimental completion date:	22-Feb-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, 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

A limit test with 5 animals (female RccHan: WIST(SPF) rat) was conducted. These animals were treated with Prosulfocarb/Pyroxsulam/Cloquintocet-mexyl SE (A18664A) by gavage at 2049 mg/kg body weight. The test item was applied undiluted at a concentration of 1.035 g/mL and at a dose volume of 1.98 mL/kg.

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 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.

Slight to moderate decreased activity was observed in all animals on test day 1 and persisted in two animals until test day 2. Slight to moderate poor coordination was observed in three animals on test day 1 and persisted in one animal until test day 2. A hunched posture was observed in four animals on test day 1 and persisted in two animals (nos. 3 and 5) until test day 2. Slight to marked ruffled fur was observed in all animals on test day 1 and persisted in four animals until test day 2. All clinical signs observed were reversible.

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 Prosulfocarb/Pyroxsulam/Cloquintocet-mexyl SE (A18664A) after single oral administration to female rats, observed over a period of 14 days, is:

LD_{50} (female rat): greater than 2049 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 done 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:		Prosulfocarb/Pyroxsulam/Cloquintocet-mexyl SE (A18664A)
Description:		Off white liquid
Batch number:		SMU0KP002
Purity:	content of prosulfocarb:	59.4 % w/w corresponding to 615 g/L
	content of pyroxsulam:	0.492 % w/w corresponding to 5.09 g/L
	content of cloquintocet-mexyl:	0.468 % w/w corresponding to 4.84 g/L
Correction factor for purity:		No
Density:		1035 kg/m^3
Stability of test item:		Stable under specified storage conditions
Reanalysis date:		End of May 2013 (as stated by the Sponsor) 31-May-2013 (as handled by Harlan Laboratories Ltd.)
Storage conditions:		At a temperature < 30°C (as specified by the Sponsor) At room temperature of 20±5°C (as handled by Harlan Laboratories Ltd.), light protected.
Safety precautions:		Routine hygienic procedures (gloves, goggles, face mask)

3.2 Experimental Design

The animals received a single dose of the test item by oral gavage administration after being fasted for approximately 17 to 18 hours, but with free access to water. Food was presented approximately 3 hours after dosing.

No dose formulations were prepared. The test item was administered undiluted, with respect to the density.

Dosing started in one female animal at a dose level of 2049 mg/kg. The application volume was 1.98 mL/kg body weight. Five animals were treated at this dose because no deaths occurred and no animals had to be killed during the course of the study.

Application Scheme

Animal number	Dose [mg/kg body weight]	Volume [mL/kg body weight]
1	2049	1.98
2	2049	1.98
3	2049	1.98
4	2049	1.98
5	2049	1.98

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 days 8 and 15. All animals were examined macroscopically after being sacrificed at the end of the study.

3.3 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 weeks

Body weights (at treatment):	166.5 – 194.9 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:	At least 5 days under laboratory conditions, after health examination. Only animals without any visible signs of illness were used for the study.

3.4 Husbandry

Room number:	0105 / Harlan Laboratories Ltd., Füllinsdorf
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%). 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. 67, Enviro-dri from Lillico, Biotechnology, Surrey, UK) during treatment and observation.
Diet:	Pelleted Teklad rat-mouse diet 2914C (batch nos. 68/10 and 30/10, 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 Füllinsdorf *ad libitum*. Results of bacteriological, chemical and contaminant analyses were archived at Harlan Laboratories Ltd.

3.5 *Post Mortem* Investigations

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

3.6 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 traceability.

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)

Slight to moderate decreased activity was observed in all animals on test day 1 and persisted in two animals (nos. 3 and 5) until test day 2. Slight to moderate poor coordination was observed in three animals on test day 1 and persisted in one animal (no. 3) until test day 2. A hunched posture was observed in four animals on test day 1 and persisted in two animals (nos. 3 and 5) until test day 2. Slight to marked ruffled fur was observed in all animals on test day 1 and persisted in four animals (nos. 2-5) until test day 2. All clinical signs observed had reversed by test day 2 (no. 1) or test day 3 (nos. 2-5).

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 Prosulfocarb/Pyroxsulam/Cloquintocet-mexyl SE (A18664A) after single oral administration to female rats, observed over a period of 14 days, is:

LD_{50} (female rat): greater than 2049 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	2049	15	O
2	2049	15	O
3	2049	15	O
4	2049	15	O
5	2049	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	2049	Decreased activity (3)	.	1	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Ruffled fur (3)	.	1	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	2049	Decreased activity (3)	.	.	1	1	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Hunched posture (1)	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Ruffled fur (3)	.	1	2	2	2	3	1	.	.	.	.	.	.	.	.	.	.	.	.	.
3	2049	Poor coordination (3)	.	.	1	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
		Decreased activity (3)	.	2	2	2	2	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.
		Hunched posture (1)	.	1	1	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
		Ruffled fur (3)	.	.	.	.	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
4	2049	Poor coordination (3)	.	.	1	2	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Decreased activity (3)	.	1	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Hunched posture (1)	.	1	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Ruffled fur (3)	.	.	.	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
5	2049	Poor coordination (3)	.	.	1	1	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Decreased activity (3)	.	1	1	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
		Hunched posture (1)	.	.	1	1	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
		Ruffled fur (3)	.	.	.	1	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	2049	195.2	193.2	207.4	212.5	19.3
2	2049	197.3	194.9	207.0	221.3	26.4
3	2049	187.1	177.2	191.7	202.6	25.4
4	2049	186.2	178.3	190.9	197.1	18.8
5	2049	168.5	166.5	182.4	189.9	23.4

Macroscopic Findings

Animal no.	Dose [mg/kg]	Day of mortality [test day]	Macroscopic findings
1	2049	15	No findings noted
2	2049	15	No findings noted
3	2049	15	No findings noted
4	2049	15	No findings noted
5	2049	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

A18664A

prosulfocarb/ pyroxsulam/ cloquintocet-mexyl SE(600/004.5/004.5)

SMU0KP002

Batch Identification	**SMU0KP002**
Product Code	**A18664A**
Other Product Code(s)	prosulfocarb/ pyroxsulam/ cloquintocet-mexyl SE(600/004.5/004.5)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of prosulfocarb***	**59.4 % w/w corresponding to 615 g/l**
– **Content of pyroxsulam***	**0.492 % w/w corresponding to 5.09 g/l**
– **Content of cloquintocet-mexyl***	**0.468 % w/w corresponding to 4.84 g/**

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

Methodology used for Characterization: HPLC

Physical Analysis

– **Appearance**	**off white liquid**
– **Density ***	**1035 kg/m^3**

Stability:

– **Storage Temperature**	**< 30°C**
– **Recertification Date**	**End of May 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: 122100

Authorisation:

24. Jan. 2011

Dr. P. Kundel
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