Acibenzolar-S-methyl

Acibenzolar-S-methyl FS (A21924A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Final Report

DATA REQUIREMENT(S): OECD Test Guideline 425 (2008)
EPA OPPTS 870.1100 (2002)
EC 440/2008 (2008)

AUTHOR(S): Balázs Oroszlány, DVM

COMPLETION DATE: 01 December 2016

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

LABORATORY PROJECT ID: Report Number: 16/185-001P
Study Number: 16/185-001P
Task Number: TK0151949

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

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

This page is intentionally left blank.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study has been performed in accordance with the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 42/2014. (VIII. 19.) EMMI decree of the Ministry of Human Capacities 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.

The in-life phase of the study was conducted by András Mátyás. Following the resignation of the originally appointed Study Director, I took responsibility for the study.

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: 01 December 2016

Balázs Oroszlány, DVM
Study Director

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

FLAGGING STATEMENT

This page is intentionally left blank.

QUALITY ASSURANCE STATEMENT

Study Number: 16/185-001P

Study Title: Acibenzolar-S-methyl FS (A21924A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)

Test Item: Acibenzolar-S-methyl FS (A21924A)

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
31 August 2016	Study Plan	31 August 2016	31 August 2016
01 September 2016	Treatment	01 September 2016	01 September 2016
26 September 2016	Amendment 1 to the Sudy Plan	26 September 2016	26 September 2016
18 October 2016	Draft Report	18 October 2016	18 October 2016
17 November 2016	Final Report	17 November 2016	17 November 2016

Signature: Nikolett Németh Date: 01 December 2016

Nikolett Németh, B.Sc.
QA Inspector

MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd. (as Sponsor) and CiToxLAB Hungary Ltd. (as Test Facility) the study titled "Acibenzolar-S-methyl FS (A21924A) - Acute Oral Toxicity Study in Rats (Up and Down Procedure)" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 01 December 2016

Alyson Leyshon, M.Sc.
Managing Director

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
András Mátyás, M.Sc.	Study Director (in-life phase)
Balázs Oroszlány, DVM	Study Director (reporting phase)
Viktória Zelenák, M.Sc.	Assistant Scientist
Vanda Gyimesi, M.Sc.	Director of Quality Assurance Unit
Leila Merazga, M.Sc.	Quality Assurance Unit
Nikolett Németh, B.Sc.	Quality Assurance Unit
István Pásztor, DVM	Veterinary Control
Peter Maslej, DVM, Ph.D.	Director of Pathology
Tamás Mészáros, Ph.D.	Head of Pharmacy
William Masinja, B.Sc., M.Sc.	Syngenta Study Manager

Study dates

Study Initiation Date	31 August 2016
Experimental Starting Date	01 September 2016
Experimental Completion Date	22 September 2016
Receipt of Animals	18 August 2016
Acclimatisation	At least 14 days
Treatment	01 September 2016 (female no. 2182) 06 September 2016 (female no. 2183) 08 September 2016 (female no. 2184)
Observation	01 - 15 September 2016 (female no. 2182) 06 - 20 September 2016 (female no. 2183) 08 - 22 September 2016 (female no. 2184)

Deviation from the guidelines

Relative humidity values (maximum of 75%) outside the expected ranges of 30-70% were recorded during the study. However, these minor differences of the environmental parameters were considered not to adversely affect the results of integrity of the study as confirmed by the clinical veterinarian.

In the Study Plan, an inappropriate dosing scheme was indicated for a limit test with 5000 mg/kg bw, suggesting 5 animals for the limit test. However, the OECD 425 guideline requires only 3 animals, if there is no mortality. The in life stage of the study followed the OECD 425 guideline, using 3 animals, therefore this deviation is considered not to affect the validity or outcome of the study.

Performing laboratory test substance reference number
160151

Other
The study documents and samples:
- study plan and amendment,
- 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 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

In this acute oral toxicity study, 3 female Crl:WI rats were given a single oral (gavage) dose of acibenzolar-S-methyl FS (A21924A) at the dose level of 5000 mg/kg body weight (bw). The animals were fasted overnight prior to treatment and food was returned 3 hours after dosing.

Individual animals were dosed sequentially with at least 48-hour intervals. The time intervals between doses were determined by the onset, duration and severity of clinical signs. The first animal was treated at a dose level of 5000 mg/kg bw. The dose selection for the next animal followed the recommendation of AOT425StatPgm software, based on results available.

Animals were observed individually after dosing at 30 minutes, then 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), just before dosing (on Day 0) and weekly thereafter or at death. All surviving animals were euthanized and all study animals examined macroscopically at the end of the observation period or at death.

1.2 Results

No mortality occurred at 5000 mg/kg bw.

Animals were symptom free during the treatment.

There were no treatment related effects on body weight or body weight gain; however slight bodyweight loss was recorded during the second week in one animal. Body weights were within the range commonly recorded for this strain and age.

No external findings or internal changes were observed in any study animals at necropsy.

1.3 Conclusion

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of the test item, acibenzolar-S-methyl FS (A21924A), was greater than 5000 mg/kg bw in female Crl:WI rats.

2.0 INTRODUCTION

2.1 Purpose

The purpose of the study was to assess the oral toxicity of the test item acibenzolar-S-methyl FS (A21924A) 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 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.

- Commission Regulation (EC) No. 440/2008 of 30 May 2008, B.1.TRIS

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

The following information was provided by the Sponsor.

Name:	Acibenzolar-S-methyl FS (A21924A)
Other product name:	CGA245704 FS (375)
Batch number:	SMU6BL002
Product code:	A21924A
Active ingredient*:	Acibenzolar-S-methyl – 32.9 % w/w corresponding to 380 g/L
Appearance:	Yellow liquid
Density:	1155 kg/m^3
Recertification date:	28 February 2019
Storage conditions:	Room temperature (<30^oC)
Safety precautions:	Routine safety precautions (gloves, goggles, face mask, lab coat) for unknown materials were applied to assure personnel health and safety.

*: No correction for purity was applied.

The Certificate of Analysis is presented in Appendix 2.

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor.

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 Pharmacy of CiToxLAB Hungary Ltd. on the basis of the information provided by Sponsor.

3.1.2 Formulation

Test item was used undiluted at the dose level of 5000 mg/kg bw.

3.2 Experimental Design

3.2.1 Animals

Species and strain:	Crl:WI Wistar rats
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sandhofer Weg 7, D-97633 Sulzfeld, Germany
Hygienic level:	SPF at arrival, standard housing conditions during study
Justification of strain:	Recognized by international guidelines as a recommended test system.
Number of animals:	3
Sex:	Female rats, nulliparous and non-pregnant.
Age when treated:	Young adult rats, 9-10 weeks old.
Body weight (at dosing):	214-222 g
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.
Randomization:	Selected by hand at time of delivery.
Acclimatization time:	At least 14 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:	522/9
Housing / Enrichment:	Animals were housed individually in Type II polypropylene/polycarbonate cages. Rodents were housed with deep wood sawdust bedding to allow digging and other normal rodent activities.
Bedding:	Lignocel 3/4-S Hygienic Animal Bedding (produced by J. Rettenmaier & Söhne GmbH + Co.KG, Germany) was available to animals during the study.
Nesting:	ARBOCEL® nest building material (produced by J. Rettenmaier & Söhne GmbH + Co.KG, Germany) was available to animals during the study.
Light:	12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature:	20.8 – 23.7 °C
Relative humidity:	32 – 75 %
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

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 main data of the lot used in the study was: Batch number: 278 5652, Expiry date: November 2016. The food was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study. Details of the diet are archived with the raw data at CiToxLAB Hungary Ltd.

3.2.4 Water supply and quality control

Animals received tap water from the municipal supply from 500 mL bottles *ad libitum*. The water was fit for human consumption and was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

Water 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 street 36., Hungary). The quality control results are retained in the archive at 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 3 animals (female Crl:WI rats). A limit dose of 5000 mg/kg bw was selected by the Study Director after discussion with the Sponsor. The density of the test item is 1155 kg/m^3 as provided by the Sponsor, therefore the dose volume was 4.33 mL/kg bw.

The individual dose volumes used are shown in the following table.

Animal Number	Dose [mg/kg body weight]	Volume Dosed [mL]	Bodyweight [g]	Mortality
2182	5000	0.95	219	Survived
2183	5000	0.93	214	Survived
2184	5000	0.96	222	Survived

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 animals were fasted overnight prior to treatment. Water was still available, *ad libitum* overnight. Animals were weighed before dosing and the food was returned 3 hours after the treatment.

Individual animals were dosed sequentially following an interval of at least 48 hours.

3.4 Observations

3.4.1 Clinical observations

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.

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

3.4.2 Body weight measurement

The body weights were recorded on Days -1(prior to removal of food), 0 (before treatment), 7 and 14.

3.5 *Post Mortem* Investigations

All animals were subjected to gross macroscopic evaluation. All survived animals were euthanized under pentobarbital anaesthesia (Euthanimal 40%, details in 3.5.1) at the end of the observation period. After examination of the external appearance, 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.

3.5.1 Material used for euthanasia

Name:	Euthanimal 40% (Pentobarbital sodium)
Lot No.:	1409236-06
Expiry Date:	September 2017
Produced by:	AlfasanNederland BV, Kuipersweg 9, 3449 JA Woerden, The Netherlands

3.6 Data Evaluation

The type, severity and duration of clinical observations are described in the tables and results of this document. Body weight and body weight changes are summarised in tabular form. Necropsy findings are described and summarised in tabular form.

The LD_{50} was calculated using the AOT425StatPgm program. This program was prepared for the US Environmental Protection Agency by Westat, May 2001 and updated by the US EPA June 2003. This programme was constructed using the most appropriate method to estimate the LD_{50}.

4.0 RESULTS AND DISCUSSION

4.1 Mortality

No mortality occurred at 5000 mg/kg bw during the study.

4.2 Clinical Signs

All animals were symptom free.

Individual clinical observations and mortality results are presented in Table 1.

4.3 Body Weights

There were no treatment related effects on body weight or body weight gain changes; however, slight bodyweight loss was recorded during the second week in one animal. 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

No external findings or internal changes were noted at necropsy.

Macroscopic findings 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, acibenzolar-S-methyl FS (A21924A), was greater than 5000 mg/kg bw in female Crl:WI rats.

TABLES SECTION

TABLE 1 Individual Findings – Clinical Signs

DOSE LEVEL: 5000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Observations	Observation days												Frequency
			0						1	2	3	4	5	6	7-14	
			30'	1h	2h	3h	4h	6h								
1	2182	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
2	2183	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20
3	2184	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	20/20

Remarks: + = present

h = hour (s) ' = minute

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

TABLE 2 Body Weight and Body Weight Gain

DOSE LEVEL: 5000 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	2182	233	219	249	261	-14	30	12	28
2	2183	230	214	246	240	-16	32	-6	10
3	2184	233	222	249	272	-11	27	23	39
Mean:		232.0	218.3	248.0	257.7	-13.7	29.7	9.7	25.7
Standard deviation:		1.7	4.0	1.7	16.3	2.5	2.5	14.6	14.6

TABLE 3 Macroscopic Findings

DOSE LEVEL: 5000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Necropsy Date/ Necropsy Day	External Observations	Internal Observations	Organ/Tissue
1	2182	15 September 2016 Day 14	No external observations recorded	No internal observations recorded	Not applicable
2	2183	20 September 2016 Day 14	No external observations recorded	No internal observations recorded	Not applicable
3	2184	22 September 2016 Day 14	No external observations recorded	No internal observations recorded	Not applicable

APPENDICES SECTION

APPENDIX 1 Pathology Report

Study code. 16/185-001P

PATHOLOGY REPORT

Introduction

The objective of the study was to assess the acute oral toxicity of acibenzolar-S-methyl FS (A21924A) when administered in a single dose to rats at a dose level of 5000 mg/kg bw.

Methods

All 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.

TERMINAL (DAY 14)

Macroscopic Findings

There was no evidence of the macroscopic observations in animals terminated on Day 14.

Conclusion

A single oral gavage of acibenzolar-S-methyl FS (A21924A) to Crl: WI female rats dosed at 5000 mg/kg bw followed by the termination on Day 14 day, did not produce any gross findings.

Peter Maslej, D.V.M., Ph.D.
Director of Pathology

22 NOV 2016
Date

APPENDIX 2 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

A21924A
acibenzolar-S-methyl FS (375)
SMU6BL002

Batch Identification	**SMU6BL002**
Product Code	**A21924A**
Other Product Code(s)	CGA245704 FS (375)

Chemical Analysis
(Active Ingredient Content)

- **Identity of the Active Ingredient(s)*** **confirmed**
- **Content of acibenzolar-S-methyl*** **32.9 % w/w corresponding to 380 g/l**

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

Methodology used for Characterization / Recertification: HPLC, oscillating density meter

Physical Analysis

- **Appearance** **yellow liquid**
- **Density*** **1155 kg/m^3**

Stability:

- **Storage Temperature** **< 30°C**
- **Recertification Date** **End of February 2019**

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: CHMU160097
Study number(s) of batch recertification: ---

Authorization: **9 March 2016**

Daniel Jenniches
Analytical Development & Product Chemistry

APPENDIX 3 GLP Certificate

OGYÉI
National Institute of
Pharmacy and Nutrition

H-1051 Budapest, Zrínyi u. 3.
1372 P.O. Box:450.
Tel: +36 1 88 69-300, Fax: +36 1 88 69 460
E-mail: ogyei@ogyei.gov.hu, Web: www.ogyei.gov.hu

Ref. no: OGYI/19440-7/2015
Admin.: Szatmári Andrea
Date: 22 September, 2015

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, in vitro studies and 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: **02-04. June 2015.**

Dr. József Reiter
Deputy Director-General

Note: Translation of the Stamp on the official document ("Országos Gyógyszerészeti és Élelmezés-egészségügy Intézet"): ("National Institute of Pharmacy and Nutrition")