RCC Study Number B35493

Syngenta Task No: T013089-05

NOA449280:

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

Report

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1 PREFACE

1.1 GENERAL

Title	NOA449280: Acute Oral Toxicity Study in the Rat (Up and Down Procedure)
Sponsor	Syngenta Ltd Alderley Park Macclesfield Cheshire, SK10 4TJ United Kingdom
Monitoring Scientist	Mr. Dave Lees
Test Facility	RCC Ltd Wölferstrasse 4 4414 Füllinsdorf / Switzerland

1.2 RESPONSIBILITIES

Study Director	G. Arcelin
Deputy for Study Director	Dr. C. Simon
Technical Coordinators	F. Frickert / M. Bernstein
Head of RCC Quality Assurance	I. Wüthrich

1.3 SCHEDULE

Experimental Starting Date	17-APR-2007
Experimental Completion Date	16-MAY-2007
Delivery of Animals	17-APR-2007 (female no. 1) 19-APR-2007 (female no. 2) 25-APR-2007 (female no. 3)
Acclimatization	17-APR-2007 to 23-APR-2007 (female no. 1) 19-APR-2007 to 25-APR-2007 (female no. 2) 25-APR-2007 to 01-MAY-2007 (female no. 3)

Treatment	24-APR-2007 (female no. 1) 26-APR-2007 (female no. 2) 02-MAY-2007 (female no. 3)
Observation	17-APR-2007 to 08-MAY -2007 (female no. 1) 19-APR-2007 to 10-MAY-2007 (female no. 2) 25-APR-2007 to 16-MAY-2007 (female no. 3)
Study Completion Date	

1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, raw data, sample of test item(s) and the final report of the present study for a minimum of five years. Thereafter, all items described above must be archived for at least a further five years. In agreement with the Sponsor, this may be at RCC Ltd or at another GLP compliant archive facility. A report amendment need only be written if the archived items are transferred to another facility.

The report with original signatures which will be archived at RCC is the reference document. No data will be discarded without the Sponsor’s written consent.

1.5 SIGNATURE PAGE

Study Director: G. Arcelin

date: 05-JUL-2007

Management: (for) Dr. H. Fankhauser

date: 04-JUL-2007

1.6 QUALITY ASSURANCE GLP TOXICOLOGY

RCC Ltd, Toxicology, CH-4452 Itingen / Switzerland

STATEMENT

RCC STUDY NUMBER	:	B35493
TEST ITEM	:	NOA449280
STUDY DIRECTOR	:	G. Arcelin
TITLE	:	NOA449280: Acute Oral Toxicity Study in the Rat (Up and Down Procedure)

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically audited. 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-APR-2007 Study Plan	17-APR-2007
24-APR-2007 Process Based (Test System, Test Item, Raw Data, Dose Preparation, Treatment)	24-APR-2007
07-JUN-2007 Report	07-JUN-2007

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

Quality Assurance: S. van Dongen

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE

RCC STUDY NUMBER	:	B35493
TEST ITEM	:	NOA449280
STUDY DIRECTOR	:	G. Arcelin
TITLE	:	NOA449280: Acute Oral Toxicity Study in the Rat (Up and Down Procedure)

All data collected on the first day of acclimatization before the study plan was signed are excluded from this statement.

The stability of the test item dilutions under the test conditions is unknown. Therefore, it is excluded from this statement.

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

Study Director:

G. Arcelin

.....

date: 05-JUL-2007

1.8 TEST GUIDELINES

The study procedures described in this report meet or exceed the requirements of the following guidelines:

OECD guideline reference 425 (2001): Acute Oral Toxicity - Up-and-Down Procedure.

Japanese MAFF Test Data for Registration of Agricultural Chemicals, Test Guidelines, Acute oral toxicity studies, 12 NohSan No. 8147, Agricultural Production Bureau, November 24, 2000 [English translation by IAI:ACIS, revised on June 26, 2001 (13 Seisan No. 1739) and December 10, 2002 (14 Seisan No. 7269)].

EPA Health Effects Test Guidelines, OPPTS 870.1100 Acute Oral Toxicity EPA 712-C-03-190, December 2002.

1.9 ANIMAL WELFARE

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

1.10 REFERENCES

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

Acute Oral Toxicity (OECD Test Guideline 425) Statistical Programme (AOT 425 Stat Pgm). Version: 1.0, 2001. [http://www.oecd.org/pages/home/display general/0,3380,EN-document-524-nodirectorite-0-24-6775-8,FF.html).

2 SUMMARY

A limit test with 3 animals (female HanRcc:WIST (SPF) rat) was conducted. These animals were treated with NOA449280 by gavage at the limit dosage (5000 mg/kg body weight). The test item was diluted in vehicle (1% CMC in purified water) at a concentration of 0.25 g/mL and administered at a dosing volume of 20 mL/kg.

Table 1: Application scheme for limit test

Animal Number	Dosage [mg/kg body weight]	Volume [mL/kg body weight]
1	5000	20
2	5000	20
3	5000	20

The animals were examined daily during the acclimatization period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2-15. Mortality/viability was recorded once during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15. Body weights were recorded on day –1 (prior to removal of food), day 1 (prior to administration) and on days 8 and 15. All animals were necropsied and examined macroscopically.

All animals survived until the end of the study period.

Slightly ruffled fur was noted in all animals from the 30 minutes or 1-hour reading to test day 2, 3 or 4. Hunched posture was noted in all animals from the 1- or 2-hour to the 3- or 5-hour reading. Slight sedation was recorded for one animal from the 1- to 5-hour reading.

The body weight of the animals was within the range commonly recorded for this strain and age.

No macroscopic findings were recorded at necropsy.

3 CONCLUSION

The median lethal dose of NOA449280 after single oral administration to female rats, observed over a period of 14 days is:

LD_{50} (female rat): greater than 5000 mg/kg body weight

4 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).

5 MATERIALS AND METHODS

5.1 TEST SYSTEM

Test system	Rat, HanRcc:WIST (SPF)
Rationale	Recognized by the international guidelines as a recommended test system.
Source	RCC Ltd, Laboratory Animal Services CH-4414 Füllinsdorf / Switzerland
Number of animals per group	One female
Total number of animals	3 females
Age when treated	11-12 weeks
Identification	Unique cage number and corresponding color-coded spots on the tail. The animals were marked at acclimatization start.
Randomization	Randomly selected by hand at time of delivery.
Acclimatization	Under laboratory conditions, after health examination. Only animals without any visible signs of illness were used for the study.

5.2 HUSBANDRY

Room no.	0105 / RCC Ltd, Füllinsdorf
Conditions	Standard Laboratory Conditions. Air-conditioned with 10-15 air changes per hour, and continuously monitored environment with ranges for room temperature 22 ± 3 °C and for relative humidity between 30-70 % (values above 70 % during cleaning process possible), automatically controlled light cycle of 12 hours light and 12 hours dark, music during the daytime light period.
Accommodation	Individually in Makrolon type-3 cages with standard softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz) during treatment and observation.

Diet	Pelleted standard Provimi Kliba 3433 rat/mouse maintenance diet, batch no. 89/06 (Provimi Kliba AG, CH-4303 Kaiseraugst/Switzerland) *ad libitum*. Results of analyses for contaminants are archived at RCC Ltd.
Water	Community tap water from Füllinsdorf *ad libitum*. Results of bacteriological, chemical and contaminant analyses are archived at RCC Ltd.

5.3 TEST ITEM

The following information was provided by the sponsor:

Identification	NOA449280
CAS No.	352010-68-5
Description	Solid; beige powder
Batch number	SEZ3AP006/MILLED
Purity	94.5%
Stability of test item	Stable under storage conditions.
Stability of test item dilution	Unknown in 1% CMC; is excluded from the statement of compliance
Reanalysis date	31-MAR-2011
Storage conditions	At a temperature < 30°C; light protected, dry place
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

5.4 VEHICLE

The vehicle was chosen by the sponsor. No formulation trial was performed for 1% CMC.

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

Identification	CMC
Description	White powder
Batch number	1119535 22105125
Source	FLUKA Chemie AG, CH-9471 Buchs
Stability of vehicle	Stable under storage conditions; expiration date: August 2007
Storage conditions	At room temperature (range of 20 ± 5 °C), light protected.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

5.5 DOSE FORMULATION

Dose levels are in terms of the test item as supplied by the sponsor.

The dose formulations were made shortly before each dosing occasion using a magnetic stirrer and a spatula as homogenizers.

The test item was ground using a mortar and a pestle. Thereafter, the test item was weighed into a tared glass beaker on a suitable precision balance and the vehicle added (weight:volume).

Homogeneity of the test item in the vehicle was maintained during administration using a magnetic stirrer.

5.6 TREATMENT

Three animals received a single dose of the test item at 5000 mg/kg body weight by oral gavage administration after being fasted for approximately 16 to 19 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing.

The application volume was 20 mL/kg.

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

5.7 OBSERVATIONS

Mortality / Viability	Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1 (with the clinical signs) and twice daily during days 2-15.
Body weights	On test day –1 (prior to removal of food), on test days 1 (prior to administration), 8 and 15.
Clinical signs	Daily during the acclimatization period, during the first 30 minutes and at approximately 1, 2, 3 and 5 hours after administration on test day 1. Once daily during days 2-15. All abnormalities were recorded.

6 PATHOLOGY

6.1 NECROPSY

All animals were killed at the end of the observation period by Carbon dioxide asphyxiation and discarded after macroscopic examinations were performed. No organs or tissues were retained.

7 STATISTICAL ANALYSIS

The statistical programme (AOT 425 Stat Pgm) version: 1.0, 2001. [http://www.oecd.org/pages/home/display general/0,3380,EN-document-524-nodirectorite-0-24-6775-8,FF.html) was used for the selection of dose levels and calculation of the LD_{50} values.

8 DATA COMPILATION

Body weights were recorded on-line.

Clinical signs were recorded on data sheets.

Mortality/viability were compiled into the RCC Tox Computer System during recording and/or recorded on data sheets.

Macroscopic findings were compiled into the RCC Tox Computer System during recording.

The RCC Tox Computer System (RCC-Tox-Lims) had been 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).

9 RESULTS

9.1 MORTALITY

No deaths occurred during the study.

9.2 CLINICAL SIGNS

Slightly ruffled fur was noted in all animals from the 30 minutes or 1-hour reading to test day 2, 3 or 4. Hunched posture was noted in all animals from the 1- or 2-hour to the 3- or 5-hour reading. Slight sedation was recorded for one animal from the 1- to 5-hour reading.

9.3 BODY WEIGHTS

The body weight of the animals was within the range commonly recorded for this strain and age.

9.4 MACROSCOPIC FINDINGS

No macroscopic findings were recorded at necropsy.

9.5 MEDIAN LETHAL DOSE

The median lethal dose of NOA449280 after single oral administration to female rats, observed over a period of 14 days is:

LD_{50} (female rat): greater than 5000 mg/kg body weight

10 INDIVIDUAL FINDINGS

10.1 MORTALITY / CLINICAL SIGNS

Dose mg/kg bw	Ani-mal No.	Sex	Signs	Test days																	
				1					2	3	4	5	6	7	8	9	10	11	12	13	14	15
				0.5*	1*	2*	3*	5*														
5000	1	F	No clinical signs	√								√	√	√	√	√	√	√	√	√	√	√
			Ruffled fur		1	1	1	1	1	1	1											
			Hunched posture		√	√	√	√														
			Sedation		1	1	1	1														
5000	2	F	No clinical signs	√							√	√	√	√	√	√	√	√	√	√	√	√
			Ruffled fur		1	1	1	1	1	1												
			Hunched posture			√	√	√														
5000	3	F	No clinical signs							√	√	√	√	√	√	√	√	√	√	√	√	√
			Ruffled fur	1	1	1	1	1	1													
			Hunched posture		√	√	√															

Key: 1 slight, √ noted
* Examinations were performed during the first 30 minutes and approximately 1, 2, 3 and 5 hours after treatment

No clinical signs were evident in any animal during the acclimatization period.

10.2 BODY WEIGHTS

Dose mg/kg bw	Animal No.	Sex	Day -1 (prior to removal of food)	Day 1 (prior to treatment)	Day 8	Day 15
5000	1	F	198.9	193.2	204.5	213.2
5000	2	F	203.0	200.7	217.0	231.8
5000	3	F	182.1	172.8	189.2	196.7

Body weights are presented in grams.

10.3 MACROSCOPIC FINDINGS

Dose mg/kg body weight	Animal No.	Sex	Mode of death	Findings
5000	1	F	S	No macroscopic findings
5000	2	F	S	No macroscopic findings
5000	3	F	S	No macroscopic findings

S: scheduled necropsy

11 CERTIFICATE OF ANALYSIS

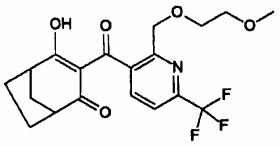
syngenta

GLP Testing Facility WMU
Analytical Development &
Product Chemistry GS2131

Syngenta Crop Protection Münchwilen AG
Breitenloh 5
CH-4333 Münchwilen

Certificate of Analysis

NOA449280



SEZ3AP006/MILLED - Purity 94.5 % w/w

Batch Identification	**SEZ3AP006/MILLED**
Product Code	**NOA449280**
Other Product Code(s)	---
ISO Common Name	NOA449280
CA Reg. No.	352010-68-5
CA Index Name	bicyclo[3.2.1]oct-3-en-2-one, 4-hydroxy-3-[[2-[(2-methoxyethoxy)methyl]-6-(trifluoromethyl)-3-pyridinyl]carbonyl]-
IUPAC Name	4-hydroxy-3-[2-(2-methoxy-ethoxymethyl)-6-(trifluoromethyl)-pyridine-3-carbonyl]-bicyclo[3.2.1]oct-3-en-2-one
Molecular formula	C_{19} H_{20} F_3 N O_5
Molecular mass	399.4
Chemical Analysis	
– **Identity***	**confirmed**
– **Content of NOA449280 ***	**94.5 % w/w**
Methodology used for Characterization / Reanalysis	HPLC
Physical Analysis	
– **Appearance ***	**brown beige powder**
Stability:	
– **Storage Temperature**	**< 30°C**
– **Reanalysis date**	**March 2011**

The stability of this test substance will be controlled by reanalysis of material held in the inventory at Syngenta Crop Protection Muenchwilen AG at the appropriate time.

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.

Characterisation: 110450 Reanalysis: 117332

Authorisation:

March 06, 2007 i.V.

Dr. P. Kundel
Analytical Development & Product Chemistry

12 GLP – CERTIFICATION

The Swiss GLP Monitoring Authorities

Swiss Federal Office of Public Health

Swiss Agency for the Environment, Forests and Landscape

Swissmedic Swiss Agency for Therapeutic Products

Statement of GLP Compliance

It is hereby confirmed that

during the period of — April 22, 25 – 29, 2005
May 09 – 13, 2005

the following Facilities of — **RCC Ltd**
4452 Itingen
Switzerland

were inspected by the Federal Office of Public Health, the Swiss Agency for Therapeutic Products and the Swiss Agency for the Environment, Forests and Landscape with respect to the compliance with the Swiss legislation on Good Laboratory Practice.

Facilities	**Areas of expertise ***
- Test Facility: Toxicology	**TOX, ACC, OTH (Safety Pharmacology, Alternative Test Systems)**
- Test Facility: Environmental Chemistry & Pharmanalytics	**ACC, ECT, ENF, EMN, PCT, RES, OTH (Animal Metabolism)**
- Archive Facilities	

The inspections were performed in agreement with the OECD Guidelines for National GLP Inspections and Audits. It was found that the aforementioned test facilities were operating in compliance with the Swiss Ordinance relating to Good Laboratory Practice [RS 813.016.5] at the time they were inspected.

Federal Office of Public Health
The Director

Bern, November 2005

Prof. Th. Zeltner

* TOX = Toxicology ; ACC = Analytical and Clinical Chemistry ; ECT = Environmental toxicity on aquatic and terrestrial organisms ; ENF = Behaviour in water, soil and air. Bioaccumulation ; EMN = Studies on effects on mesocosms and natural ecosystems; PCT = Physical-chemical testing ; RES = Residue studies ; OTH = Other, to be specified.