RCC Study Number B19697

Syngenta Task Number: T005338-06

Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B):

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

Report

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United Kingdom

RCC

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1 PREFACE

1.1 GENERAL

Title	Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B): 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	27-FEB-2007
Experimental Completion Date	03-APR-2007
Delivery of Animals	27-FEB-2007 (female no. 1) 01-MAR-2007 (female no. 2) 06-MAR-2007 (female no. 3) 08-MAR-2007 (female no. 4) 13-MAR-2007 (female no. 5)
Acclimatization	27-FEB-2007 to 05-MAR-2007 (female no. 1) 01-MAR-2007 to 07-MAR-2007 (female no. 2) 06-MAR-2007 to 12-MAR-2007 (female no. 3) 08-MAR-2007 to 14-MAR-2007 (female no. 4) 13-MAR-2007 to 19-MAR-2007 (female no. 5)

Treatment	06-MAR-2007 (female no. 1) 08-MAR-2007 (female no. 2) 13-MAR-2007 (female no. 3) 15-MAR-2007 (female no. 4) 20-MAR-2007 (female no. 5)
Observation	27-FEB-2007 to 20-MAR-2007 (female no. 1) 01-MAR-2007 to 22-MAR-2007 (female no. 2) 06-MAR-2007 to 14-MAR-2007 (female no. 3) 08-MAR-2007 to 29-MAR-2007 (female no. 4) 13-MAR-2007 to 03-APR-2007 (female no. 5)

1.4 ARCHIVING

RCC Ltd (CH-4452 Itingen / Switzerland) will retain the study plan, amendment, 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: 26-JUL-2007

Management: Dr. H. Fankhauser

date: 26-JUL-2007

1.6 QUALITY ASSURANCE GLP TOXICOLOGY

RCC Ltd, Toxicology, CH-4452 Itingen / Switzerland

STATEMENT

RCC STUDY NUMBER	:	B19697
TEST ITEM	:	Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B)
STUDY DIRECTOR	:	G. Arcelin
TITLE	:	Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B): 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, with exception of the formulation trials, 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
23-FEB-2007 Study Plan	23-FEB-2007
13-MAR-2007 Process Based (Test System, Test Item, Raw Data, Dose Preparation, Treatment)	13-MAR-2007
07-JUN-2007 Draft Report	07-JUN-2007
25-JUL-2007 Report	25-JUL-2007

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

Quality Assurance: S. van Dongen

S. van Dongen

date: 26-Jul-2007

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE

RCC STUDY NUMBER	:	B19697
TEST ITEM	:	Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B)
STUDY DIRECTOR	:	G. Arcelin
TITLE	:	Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B): Acute Oral Toxicity Study in the Rat (Up and Down Procedure)

The formulation trials were performed before the study initiation date. Therefore, they are 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: 26-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).

1.11 SUMMARY OF STUDY PLAN AMDENDMENT

Study Plan Amendment No. 1:

Change of study director and deputy.

The test item density was corrected according to the certificate of analysis.

2 SUMMARY

A limit test with 5 animals (female HanRcc:WIST (SPF) rat) was conducted. These animals were treated with Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B) by gavage at the limit dosage of 2000 mg/kg body weight. The test item was applied undiluted as delivered by the sponsor at a dosing volume of 1.57 mL/kg.

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.

One animal died spontaneously at test day 2. All other animals survived until the end of the study period.

All the animals showed a slightly ruffled fur at the 30-minute or 1-hour reading. The ruffled fur persisted until test day 7, 9 or 12. A slight to moderate sedation was noted in all animals from the 1- or 2-reading until 3 hours, 5 hours post-dose or on test day 2. Two hours after application four animals had watery feces which were present in 3 animals until 5 hours after treatment. The feces were soft in one animal at test day 2 and 3. Hunched posture was observed in all 5 animals and was distributed between the 30-minute post-dose to test day 3.

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

The animal which died spontaneously showed a distended stomach with liquid contents, empty jejunum and ileum and not collapsed lungs. No macroscopic findings were recorded in the other animals at the scheduled necropsy.

3 CONCLUSION

The median lethal dose of Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B) 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

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	5 females
Age when treated	11 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 nos. 80/06 and 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	Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B)
Description	Light beige liquid
Density	1.26 g/mL
Batch number	PHY6A60099
Purity / Formulation (Active ingredient content)	Content of azoxystrobin 96.3 g/l corresponds to 7.63 % w/w Content of chlorothalonil 501 g/l corresponds to 39.7 % w/w
Stability of test item	Stable under storage conditions.
Reanalysis date	January 2009
Storage conditions	< 30°C, protected from light and humidity.
Safety precautions	Routine hygienic procedures were used to ensure the health and safety of the personnel.

5.4 DOSE FORMULATION

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

The test item was applied undiluted as delivered by the sponsor.

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

5.5 TREATMENT

Five animals received a single dose of the test item at 2000 mg/kg body weight by oral gavage administration after being fasted for approximately 17 to 18 hours (access to water was permitted). Food was provided again approximately 3 hours after dosing.

The application volume was 1.57 mL/kg (x 1.27* g/mL = 2000 mg/kg).

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

* A density of 1.26 g/mL should have been used according to the certificate of analysis (see on page 17) and the study plan amendment No.1 to adjust the application volume at 1.58 mL.

5.6 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

The animal which died spontaneously on test day 2 was necropsied as soon as it was found dead.

All the surviving 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

One animal died spontaneously on test day 2. All other animals survived until the end of the study period.

9.2 CLINICAL SIGNS

All the animals showed a slightly ruffled fur at the 30-minute or 1-hour reading. The ruffled fur persisted until test day 7, 9 or 12. A slight to moderate sedation was noted in all animals from the 1- or 2-reading until 3 hours, 5 hours post-dose or on test day 2. Two hours after application four animals had watery feces which were present in 3 animals until 5 hours after treatment. The feces were soft in one animal at test day 2 and 3. Hunched posture was observed in all 5 animals and was distributed between the 30-minute post-dose to test day 3.

9.3 BODY WEIGHTS

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

9.4 MACROSCOPIC FINDINGS

The animal which died spontaneously showed a distended stomach with liquid contents, empty jejunum and ileum and not collapsed lungs. No macroscopic findings were recorded in the other animals at the scheduled necropsy.

9.5 MEDIAN LETHAL DOSE

The median lethal dose of Azoxystrobin/ Chlorothalonil 100/500g/l SC formulation (A14110B) 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

10 INDIVIDUAL FINDINGS

10.1 MORTALITY / CLINICAL SIGNS

Dose mg/kg bw	Animal 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*														
2000	1	F	No clinical signs	√													√	√	√	√	√	√
			Ruffled fur		1	1	1	1	1	1	1	1	1	1	1	1						
			Sedation			1	1	1														
			Watery feces			√	√	√														
			Hunched posture			√	√	√	√													
2000	2	F	No clinical signs	√																√	√	√
			Ruffled fur		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
			Sedation			1	1	1	1													
			Watery feces			√	√	√														
			Hunched posture			√	√	√	√	√												
2000	3	F	Ruffled fur		1	1	1	1	+													
			Sedation			1	1	1														
			Watery feces			√	√	√														
			Hunched posture	√	√	√	√	√														
2000	4	F	No clinical signs																	√	√	√
			Ruffled fur	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
			Sedation		2	2	2															
			Watery feces			√																
			Hunched posture	√	√	√	√	√														
2000	5	F	No clinical signs												√	√	√	√	√	√	√	√
			Ruffled fur	1	1	1	1	1	1	1	1	1	1	1								
			Sedation		1	1	1	1														
			Soft feces						√	√												
			Hunched posture	√	√	√	√	√	√													

Key: 1 slight, 2 moderate, + found dead, √ noted.
* Examinations were performed approximately 0.5, 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
2000	1	F	191.1	191.9	207.1	222.2
2000	2	F	194.1	184.3	194.5	206.2
2000	3	F	211.4	200.7	--	--
2000	4	F	189.6	178.7	200.4	214.9
2000	5	F	185.6	180.6	195.1	201.8

Body weights are presented in grams.

10.3 MACROSCOPIC FINDINGS

Dose mg/kg body weight	Animal No.	Sex	Mode of death	Findings
2000	1	F	S	No macroscopic findings
2000	2	F	S	No macroscopic findings
2000	3	F	D	Lungs: not collapsed Stomach: distended, liquid contents Jejunum/ileum: empty
2000	4	F	S	No macroscopic findings
2000	5	F	S	No macroscopic findings

S: scheduled necropsy; D: found dead

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

A14110B

azoxystrobin/chlorothalonil SC (100/500)

PHY6A60099

Batch Identification	**PHY6A60099**
Product Code	**A14110B**
Other Product Code(s)	azoxystrobin/chlorothalonil SC (100/500)
Chemical Analysis (Active Ingredient Content)	
– **Identity of the Active Ingredients ***	**confirmed**
– **Content of azoxystrobin ***	**98.3 g/l, corresponds to 7.63 % w/w**
– **Content of chlorothalonil ***	**501 g/l, corresponds to 39.7 % w/w**

Methodology used for Characterization: cap. GC

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

Physical Analysis	
– **Appearance**	**light beige liquid**
– **Density ***	**1262 kg/m^3**

Stability:	
– **Storage Temperature**	**< 30°C**
– **Reanalysis Date**	**January 2009**

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.

Characterization: 117178

Authorization:

23-Feb-2007

Dr. R. Kettner
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 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.