CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

CTL/AR7254/REGULATORY/REPORT

CGA 184927/NOA 407855 EC 200 (A13833B): ACUTE ORAL TOXICITY STUDY IN THE RAT - UP AND DOWN PROCEDURE

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STUDY DETAILS

Sponsor:	Syngenta Limited Alderley Park Macclesfield Cheshire SK10 4TJ UK
CTL Test Substance Reference Number:	Y11653/051
CTL Study Number:	AR7254

AUTHOR

I R Johnson

DATE OF ISSUE

12 February 2004

STATEMENT OF DATA CONFIDENTIALITY CLAIM

THIS DOCUMENT CONTAINS INFORMATION CONFIDENTIAL AND TRADE SECRET TO SYNGENTA LIMITED.

It should not be disclosed in any form to an outside party, nor should information contained herein be used by a registration authority to support registration of this product or any other product without the written permission of Syngenta Limited.

STATEMENT OF GLP COMPLIANCE AND AUTHENTICATION

I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated in the above study.

The study (AR7254) was conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1999, Statutory Instrument No. 3106) except for the deviation listed below. These Principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

The following GLP deviation is considered not to affect the integrity of the study or the validity of the conclusions drawn:

(i) the stability, homogeneity and achieved concentration of the test substance in the vehicle used were not determined by analysis.

I R Johnson
Study Director

..... 12 February 2004
Date

This page
may be required
by some
regulatory authorities.

QUALITY ASSURANCE STATEMENT

In accordance with CTL policy and QA procedures for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Audit/Inspection	Date of QA Report
12 Jan 2004	Draft report	16 Jan 2004
11 Feb 2004	Final report review	11 Feb 2004

In addition, inspections associated with this type of study were made as follows:

22 Jul 2003	Bodyweights, clinical observations	22 Jul 2003
30 Jul 2003	Protocol	30 Jul 2003
05 Aug 2003	Dose preparation	05 Aug 2003
02 Sep 2003	Post mortem	02 Sep 2003
11 Sep 2003	Dose administration	11 Sep 2003

Facilities and process based procedures associated with this type of study were inspected in accordance with QA Standard Operating Procedures.

So far as can be reasonably established, the methods described and the results given in the final report accurately reflect the raw data produced during the study, AR7254.

C Rooney ..... 12 February 2004

(CTL Quality Assurance Unit)

STUDY CONTRIBUTORS

The following contributed to this report in the capacities indicated:

Name	Title
I R Johnson	Study Director
D Beeston	Study Licensee
D Lees	Study Reviewer
A M Leah	Report preparation

CONTENTS

TABLES

APPENDIX

1. SUMMARY

1.1 Study design

Female Alpk:APfSD (Wistar-derived) rats received a single oral dose of 175, 550 or 2000mg/kg of CGA 184927/NOA 407855 EC 200 (A13833B). The animals were observed daily for the following 14 days for mortality and any signs of systemic toxicity.

Bodyweights were recorded at intervals during the study. At the end of the study all animals were killed and examined *post mortem*.

1.2 Results

Following a dose of 175mg/kg to one rat, the animal survived and showed no signs of systemic toxicity. The animal showed an overall bodyweight gain during the study. There were no macroscopic abnormalities at examination *post mortem*.

Following a dose of 550mg/kg to one rat, the animal survived. There were signs of slight systemic toxicity, with complete recovery by day 3. The animal showed an overall bodyweight gain during the study. There were no macroscopic abnormalities at examination *post mortem*.

Following a dose of 2000mg/kg to three rats, all the animals survived. There were signs of systemic toxicity in all animals (very minor in one animal) with complete recovery by day 7. All animals showed an overall bodyweight gain during the study. There were no macroscopic abnormalities at examination *post mortem*.

1.3 Conclusion

Based on the acute oral toxicity statistical programme (AOT 425 Stat Pgm), the acute oral median lethal dose of CGA 184927/NOA 407855 EC 200 (A13833B) was estimated to be in excess of 2000mg/kg to female rats.

2. INTRODUCTION

2.1 Purpose

The purpose of this study was to assess the acute oral toxicity of CGA 184927/NOA 407855 EC 200 (A13833B) to female rats, following a single oral dose.

The study was carried out according to the Modified Up-and-Down Procedure (ASTM, 1987).

2.2 Regulatory guidelines

The study has been done in accordance with the following Regulatory Guidelines:

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

b) United States Environmental Protection Agency, Health Effects Test Guidelines (2002), OPPTS 870.1100, Acute Oral Toxicity.

2.3 Justification for test system selection

The albino rat was used because it is the species generally recommended for the assessment of toxicity. The Alpk:APfSD strain of rat was used because of the substantial background data available for this strain, in this Laboratory, relating to studies of this type. The oral route was chosen as this represents a possible route of human exposure.

2.4 Dose level selection

Dose levels were selected using the modified up and down procedure (Appendix A).

An initial dose level of 175mg/kg was chosen using the default values in the statistical program (AOT 425 Stat Pgm). A single animal was dosed at this level. Further dose levels of 550 and 2000mg/kg were chosen using the criteria in Appendix A. A minimum of 48 hours was allowed between dosing each animal in the sequence.

2.5 Study dates

The study was initiated on 1 August 2003. The experimental phase started on 4 August 2003 and was completed on 10 September 2003.

2.6 Data storage

An original report, the study protocol and all raw data, samples and specimens, pertaining to this study are retained in the Archives, Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK.

3. TEST SUBSTANCE AND VEHICLE

3.1 Test substance

Name:	CGA 184927/NOA 407855 EC 200
Source:	Syngenta Crop Protection AG
Colour:	Clear brown
Physical state:	Liquid
Batch reference number:	SEZ2LL001
CTL test substance reference number:	Y11653/051
Formulation reference no:	A13833B
AI content of formulation (w/w):	CGA 184927 - 103g/l
	NOA 407855 - 103g/l
	CGA 185072 - 25.8g/l
Reanalysis date:	February 2005
Storage conditions:	Ambient temperature in the dark

A certificate of analysis (dated 9 April 2003) is retained in the CTL Archives. The test substance characterisation was carried out by Syngenta Crop Protection AG, Münchwilen.

3.2 Vehicle

The vehicle for the test substance was deionised water (CTL test substance reference number Y04517/015).

4. EXPERIMENTAL PROCEDURES

4.1 Dose formulations

The 175mg/kg dose (which was not corrected for the purity of the test substance) was prepared as a 35% formulation, by the CTL Central Dispensary. A measured amount of the test substance was formulated in deionised water and was thoroughly mixed. Doses of 550 and 2000mg/kg were dosed undiluted.

4.2 Analysis of dose preparation

Achieved concentration, homogeneity and stability of the (175mg/kg) dosing preparation were not determined.

4.3 Experimental design

4.3.1 Animals

Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK.
Sex/number used:	Five females
Age and weight range at start of study	Approximately 8-12 weeks old. Bodyweight 181-234g.

4.3.2 Accommodation and husbandry

Animals were individually housed, in cages suitable for animals of this strain and the weight range expected during the course of the study.

The animal room was designed to give the environmental conditions shown as follows:

Temperature:	22±3°C
Relative humidity:	30-70%
Air changes:	A minimum of 15 changes per hour
Light cycle:	Artificial, giving 12 hours light, 12 hours dark

Both temperature and relative humidity were recorded daily, with the exception (due to equipment breakdown) of one day. There was a slight increase in relative humidity from nominal (maximum 75%) on 1 day, but as the study was of such short duration this is considered to have had no detrimental effect on the scientific integrity.

Diet (RM1), supplied by Special Diets Services Limited, Witham, Essex, UK, and mains water, supplied by an automatic system, were available *ad libitum*, except that each rat was fasted overnight immediately prior to dosing to ensure its stomach was empty (the presence of food could affect the rate of absorption of the test substance).

Each batch of diet is routinely analysed for composition and for contaminants. Water is also periodically analysed for contaminants. No contaminants were found in the diet or water at levels considered likely to interfere with the purpose or outcome of the study. Certificates of analyses are retained in the CTL Archives.

4.3.3 Acclimatisation

The animals were housed under the experimental conditions for at least 5 days, prior to the start of dosing.

4.3.4 Animal identification

Animals were individually identified with a number, unique within the study, by ear punching.

A card was displayed on the front of each cage, identifying the animal within. A card was also displayed on the cage to indicate the date of the pre-dose fasting period.

4.3.5 Dose regime

Animals were dosed in the following order:

Step	Animal numbers	Dose level (mg/kg)	Comments
1	65	175	survived
2	86	550	survived
3	90	2000	survived
4	38	2000	survived
5	42	2000	survived

4.3.6 Dose administration

The volume of the dose was calculated for each animal according to its weight at the time of dosing.

A standard volume of 0.5, 0.55 or 2.0ml/kg (175, 550 and 2000mg/kg) bodyweight of the dosing preparation was administered by gavage using a stomach tube.

4.4 Clinical observations

Prior to dosing, all rats were examined to ensure that they were physically normal and behaved normally. The animals were observed for signs of systemic toxicity immediately after dosing and a further twice following dosing on day 1. Subsequent observations were made daily, up to day 15.

4.5 Bodyweights

The animals were weighed prior to fasting on the day before dosing (day -1), immediately before dosing (day 1) and on days 8 and 15. In addition, animal 90 was weighed on days 2 and 3.

4.6 Investigations *post mortem*

4.6.1 Termination

Animals were killed by an overdose of halothane vapour, followed by exsanguination.

4.6.2 Macroscopic examination

All animals were examined *post mortem*.

The examination involved an external observation and a careful examination of all thoracic and abdominal viscera. All abnormalities were recorded but tissues were not submitted for histopathological examination.

5. DATA EVALUATION

Data were evaluated using the Acute Oral Toxicity (OECD Test Guidelines 425) Statistical Programme (AOT 425 Stat Pgm).

6. RESULTS

6.1 Clinical observations and mortality (Table 1)

Following a dose of 175mg/kg to one rat, the animal survived and showed no signs of systemic toxicity.

Following a dose of 550mg/kg to one rat, the animal survived. There were signs of slight systemic toxicity, with complete recovery by day 3.

Following a dose of 2000mg/kg to three rats, all the animals survived. There were signs of systemic toxicity in all animals (very minor in one animal) with complete recovery by day 7.

6.2 Bodyweights (Table 2)

All animals showed an initial weight loss due to the pre-dose fast but all showed an overall weight gain by the end of the study.

6.3 Investigations *post mortem* (Table 3)

There were no macroscopic abnormalities at examination *post mortem*.

7. CONCLUSION

Based on the acute oral toxicity statistical programme (AOT 425 Stat Pgm), the acute oral median lethal dose of CGA 184927/NOA 407855 EC 200 (A13833B) was estimated to be in excess of 2000mg/kg to female rats.

8. 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).

GLOSSARY FOR ANIMAL DATA TABLES

NAD	-	no abnormalities detected
NO	-	number
X	-	present
S	-	slight
M	-	moderate
I	-	increased
R	-	reduced
N	-	absent
(D)	-	humanely killed on this day
wks	-	weeks
F	-	female

TABLE 1 - CLINICAL OBSERVATIONS

GROUP NO: 01 ANIMAL NO: 65 SEX: FEMALE START DATE: 5/08/03
DOSE: 175 MG/KG

CLINICAL OBSERVATION	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15
DIED ON DAY 15	----------															^
*** NAD ***	X	XXX	X	X	X	X	X	X	X	X	X	X	X	X	X	X
KILLED TERMINATION																X

TABLE 1 - CLINICAL OBSERVATIONS

GROUP NO: 02 ANIMAL NO: 86 SEX: FEMALE START DATE: 8/08/03
DOSE: 550 MG/KG

CLINICAL OBSERVATION	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15
DIED ON DAY 15	----------															^
*** NAD ***	X	X			X	X	X	X	X	X	X	X	X	X	X	X
KILLED TERMINATION																X
TIP TOE GAIT		SS	N													
UPWARD CURVATURE OF SPINE		SS	S	N												

TABLE 1 - CLINICAL OBSERVATIONS

GROUP NO: 03 ANIMAL NO: 38 SEX: FEMALE START DATE: 19/08/03
DOSE: 2000 MG/KG

CLINICAL OBSERVATION	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15
DIED ON DAY 15	----------															^
*** NAD ***	X	XX			X	X	X	X	X	X	X	X	X	X	X	X
ACTIVITY DECREASED		SS	N													
RESPONSE TO SOUND		II	I	N												
KILLED TERMINATION																X
REDUCED STABILITY		SS	N													
PILOERECTION		SS	S	N												
SIDES PINCHED IN		SS	N													
UPWARD CURVATURE OF SPINE		SS	N													
REDUCED SPLAY REFLEX		SS	S	N												
BREATHING IRREGULAR		XX	N													

TABLE 1 - CLINICAL OBSERVATIONS

GROUP NO: 03 ANIMAL NO: 42 SEX: FEMALE START DATE: 27/08/03
DOSE: 2000 MG/KG

CLINICAL OBSERVATION	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15
DIED ON DAY 15																^
*** NAD ***	X	XXXX	X	X	X	X			X	X	X	X	X	X	X	X
KILLED TERMINATION																X
REDUCED SPLAY REFLEX							M	N								

TABLE 1 - CLINICAL OBSERVATIONS

GROUP NO: 03 ANIMAL NO: 90 SEX: FEMALE START DATE: 13/08/03
DOSE: 2000 MG/KG

CLINICAL OBSERVATION	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15
DIED ON DAY 15	----------															^
*** NAD ***	X	X							X	X	X	X	X	X	X	X
ACTIVITY DECREASED		MMM	S	N		S	S	N								
MYDRIASIS (PUPIL DILATED)		XN														
KILLED TERMINATION																X
REDUCED STABILITY		SSS	N													
ABDOMINAL TONE DECREASED			S	S	S	N										
COLD							X	N								
DEHYDRATED			M	N												
PILOERECTION		SSS	M	S	X	N										
SALIVATION		SN														
SIDES PINCHED IN		MMM	M	S	S	N										
SIGNS OF SALIVATION		SS	S	N												
THIN			S	N												
UPWARD CURVATURE OF SPINE		MMM	M	S	S	S	S	N								
REDUCED SPLAY REFLEX		MMS	M	N												
BREATHING RATE		RN														

TABLE 1 - CLINICAL OBSERVATIONS

GROUP NO: 03 ANIMAL NO: 90 SEX: FEMALE START DATE: 13/08/03
DOSE: 2000 MG/KG

CLINICAL OBSERVATION	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15
DIED ON DAY 15	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	^
BREATHING IRREGULAR		X	X	N												

TABLE 2 - BODYWEIGHTS (g)

DOSE: 175 MG/KG

ANIMAL NUMBER	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15	
FEMALES																	
65	234	205	-	-	-	-	-	-	266	-	-	-	-	-	-	265	(D)

TABLE 2 - BODYWEIGHTS (g)

DOSE: 550 MG/KG

ANIMAL NUMBER	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15	
FEMALES																	
86	201	181	-	-	-	-	-	-	224	-	-	-	-	-	-	245	(D)

TABLE 2 - BODYWEIGHTS (g)

DOSE: 2000 MG/KG

ANIMAL NUMBER	DAY -1	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13	DAY 14	DAY 15	
FEMALES																	
38	181	164	-	-	-	-	-	-	211	-	-	-	-	-	-	237	(D)
42	194	176	-	-	-	-	-	-	217	-	-	-	-	-	-	236	(D)
90	195	173	165	170	-	-	-	-	207	-	-	-	-	-	-	218	(D)

TABLE 3 - MACROSCOPIC FINDINGS

ANIMAL NO: 65 SEX: F DOSE: 175 MG/KG ON STUDY: 15 days (3 wks) TERMINAL

MACROPATHOLOGY

Killed by Halothane ; NAD.

----------END OF ANIMAL----------

TABLE 3 - MACROSCOPIC FINDINGS

ANIMAL NO: 86 SEX: F DOSE: 550 MG/KG ON STUDY: 15 days (3 wks) TERMINAL

MACROPATHOLOGY

Killed by Halothane ; NAD.

----------END OF ANIMAL----------

TABLE 3 - MACROSCOPIC FINDINGS

ANIMAL NO: 38 SEX: F DOSE: 2000 MG/KG ON STUDY: 15 days (3 wks) TERMINAL

MACROPATHOLOGY

Killed by Halothane ; NAD.

----------END OF ANIMAL----------

TABLE 3 - MACROSCOPIC FINDINGS

ANIMAL NO: 42 SEX: F DOSE: 2000 MG/KG ON STUDY: 15 days (3 wks) TERMINAL

MACROPATHOLOGY

Killed by Halothane ; NAD.

----------END OF ANIMAL----------

TABLE 3 - MACROSCOPIC FINDINGS

ANIMAL NO: 90 SEX: F DOSE: 2000 MG/KG ON STUDY: 15 days (3 wks) TERMINAL

MACROPATHOLOGY

Killed by Halothane ; NAD.

----------END OF ANIMAL----------

APPENDIX A - UP-AND-DOWN METHOD : DOSING REGIME

METHOD

The initial dose level was set at 175mg/kg, since no toxicity data was available and this is the default value in the statistical program (AOT 425 Stat Pgm).

If the animal dosed at the initial level survives, a single animal is dosed at the next highest level. If the animal dosed at the initial level dies, a single animal is dosed at the next lowest level. This procedure is repeated for subsequent animals until one of the following "stopping rules" is met:

a) 3 consecutive animals survive at the limit dose (2000mg/kg)

b) 5 reversals (U turn decisions) occur in 6 consecutive animals [e.g.: 175mg/kg (animal survives), 550mg/kg (animal dies) - first U turn decision, 175mg/kg (animal survives) - second U turn decision, 550mg/kg (animal dies) - third U turn decision, 175mg/kg (animal survives) - fourth U turn decision, 550mg/kg (animal dies), fifth U turn decision, 175mg/kg (animal survives) - study complete]

c) At least 4 animals have followed the first reversal (U turn) and the specified likelihood ratios exceed the critical value.

CERTIFICATE OF ANALYSIS

syngenta

GLP Testing Facility EZA
Analytical Development &
Product Chemistry GS2131

Syngenta Crop Protection
Münchwilen AG
Breitenloh 5
CH-4333 Münchwilen

Certificate of Analysis

A13833B
CGA184927/NOA407855 EC (100/100)
& S:CGA185072 (025)
SEZ2LL001

Batch Identification	**SEZ2LL001**
Product Code	**A13833B**
Other Product Code(s)	CGA184927/NOA407855 EC (100/100) & S:CGA185072 (025)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredients ***	**confirmed**
– **Content of CGA 184927 ***	**103 g/l**
– **Content of NOA 407855 ***	**103 g/l**
– **Content of CGA 185072 ***	**25.8 g/l**
Methodology used for Characterisation	HPLC, GC

The Active Ingredients content is within the FAO limits.

Physical Analysis

– **Appearance**	**clear brown liquid**
– **Density ***	**1050 kg/m³**

Stability:

– **Storage Temperature**	**< 30°C, keep away from direct sunlight**
– **Reanalysis Date**	**February 2005**

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

This Certificate of Analysis is summarizing data which originate either from a single study or from several individual studies which have been performed in compliance with GLP. Tests marked with an asterisk (*) have been conducted within a single study/as individual studies. Raw data, documentation, study plans, any amendments to study plans and reports pertaining to this/these studie(s) are stored under the study number(s) referenced below within the archives of the GLP Testing Facility EZA at Syngenta Crop Protection Münchwilen AG. No GLP compliance is claimed for this certificate.

Characterisation: 110035

Authorisation:

09-APR-2003

Dr. Robert Kettner
Analytical Development & Product Chemistry

10006289

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