Paraquat

Paraquat SG (A19087K) - Acute Oral Toxicity in the Rat – Up-and-Down-Procedure

Final Report

DATA REQUIREMENT:	OECD No. 425 (2008) EPA OPPTS 870.1100 (2002)
AUTHOR(S):	Antony Pooles BA (Hons)
STUDY COMPLETION DATE:	12 March 2013
PERFORMING LABORATORY:	Harlan Laboratories Ltd Shardlow Business Park, Shardlow Derbyshire, DE72 2GD, United Kingdom
LABORATORY PROJECT ID:	Report Number: 41201356 Study Number: 41201356 Task Number: TK0103939
SPONSOR(S):	Syngenta Ltd Jealott's Hill International Research Centre Bracknell Berkshire RG42 6EY, United Kingdom

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

With the exception noted below the work described was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

No analysis was carried out to determine the homogeneity, concentration or stability of the test item formulation. The test item was formulated within two hours of being applied to the test system; it is assumed that the formulation was stable for this duration. This exception is considered not to affect the purpose or integrity of the study.

This report fully and accurately reflects the procedures used and data generated.

A. Pooles ..... DATE: 12/3/13 .....

Antony Pooles BA (Hons)

Study Director

Performing Laboratory: Harlan Laboratories Ltd
Shardlow Business Park, Shardlow
Derbyshire, DE72 2GD, United Kingdom

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

This study type is classed as short-term. The General Study Plan for this study type was reviewed for compliance once only on initial production. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases of this study type at least once every three months. From the 16th May 2012 the frequency of these process based inspections was changed from a monthly to a three monthly cycle. This deviation is considered not to affect the purpose or integrity of the study. In addition general facilities are inspected at least once a year and the results are reported to management.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

	24 August 2010	General Study Plan Compliance Audit
	09 May 2012	Test Item Preparation
	25 April 2012	Animal Preparation
	18 April 2012	Dosing
	08 May 2012	Assessment of Response
	10 May 2012	Necropsy
§	25 July 2012	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

..... DATE: 20 MAR 2013

For the Quality Assurance Unit*

***Authorised QA Signatures:**
Senior Audit Staff: J G Riley BSc (Hons) MRQA, J M Crowther MIScT MRQA, G Wren ONC MRQA, S Bevan BSc (Hons) MRQA, L Blaney MRQA

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Antony Pooles BA (Hons)	Study Director
Eric Yau	Syngenta Study Manager

Study dates

Study initiation date: 16 April 2012
Experimental start date: 15 May 2012
Experimental termination date: 03 July 2012

Deviations from the guidelines

None.

Retention of samples

None.

Performing Laboratory Test Substance Reference Number

171535

Other

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

TABLE OF CONTENTS

STATEMENT OF DATA CONFIDENTIALITY CLAIMS **2**

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT **3**

FLAGGING STATEMENT **4**

QUALITY ASSURANCE STATEMENT **5**

GENERAL INFORMATION **6**

TABLE OF CONTENTS **7**

1.0 EXECUTIVE SUMMARY **9**

1.1 Study Design 9

1.2 Results 9

1.3 Conclusion 10

2.0 INTRODUCTION **11**

2.1 Purpose 11

2.2 Justification for Test System 11

3.0 MATERIALS AND METHODS **11**

3.1 Test Item 11

3.2 Experimental Design 12

3.2.1 Animals 12

3.2.2 Procedure 12

3.2.3 Evaluation of Data 14

4.0 RESULTS AND DISCUSSION **14**

4.1 Mortality Data 14

4.2 Clinical Observations 14

4.3 Bodyweight 14

4.4 Necropsy 14

5.0 CONCLUSION **15**

TABLES SECTION **16**

GLOSSARY FOR TABLE 1 17

TABLE 1 Individual Clinical Observations and Mortality Data 18

TABLE 2 Individual Bodyweights and Weekly Bodyweight Changes 19

TABLE 3 Individual Necropsy Findings 20

APPENDICES SECTION **21**

APPENDIX 1 Certificate of Analysis 22

APPENDIX 2 Statement of GLP Compliance in Accordance with Directive 2004/9/EC 23

1.0 EXECUTIVE SUMMARY

1.1 Study Design

The study was performed to assess the acute oral toxicity of the test item following a single oral administration in the Wistar strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 425 "Acute Oral Toxicity – Up-and-Down-Procedure (UDP)" (adopted 03 October 2008)
- United States Environmental Protection Agency Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity, 2002

A total of six female animals were dosed individually in sequence with sufficient time (at least 48 hours) between each animal, at dose levels ranging from 55 mg/kg bodyweight to 550 mg/kg bodyweight.

The test item was administered orally as a solution in distilled water. Clinical signs and bodyweight development were monitored during the study. Animals were subjected to gross necropsy.

1.2 Results

Mortality. Mortality was observed in 2/2 animals treated at a dose level of 550 mg/kg and 1/3 animals treated at a dose level of 175 mg/kg. Two animals treated at a dose level of 175 mg/kg and the animal treated at a dose level of 55 mg/kg survived the observation period.

Clinical Observations. Signs of systemic toxicity noted were hunched posture, ataxia, pilo-erection and noisy respiration. There were no signs of systemic toxicity noted in one animal treated at a dose level of 175 mg/kg and the animal treated at a dose level of 55 mg/kg.

Bodyweight. The surviving animals showed expected gains in bodyweight over the study period.

Necropsy. Abnormalities noted at necropsy of animals that died during the study were haemorrhagic lungs, dark liver, pale spleen, dark kidneys, haemorrhagic gastric mucosa and non-glandular epithelium of the stomach and dark green coloured material or green liquid present in the stomach. No abnormalities were noted at necropsy of the remaining animals that were killed at the end of the study.

1.3 Conclusion

The acute oral median lethal dose (LD_{50}) of the test item in the female Wistar strain rat was calculated to be 175 mg/kg bodyweight (based on an assumed sigma of 0.5).

2.0 INTRODUCTION

2.1 Purpose

The study was performed to assess the acute oral toxicity of the test item following a single oral administration in the Wistar strain rat. The method was designed to meet the requirements of the following:

- OECD Guidelines for the Testing of Chemicals No. 425 "Acute Oral Toxicity – Up-and-Down-Procedure (UDP)" (adopted 03 October 2008)
- United States Environmental Protection Agency Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity, 2002

The rat was selected for this study as it is a readily available rodent species, historically used in safety evaluation studies, and is acceptable to appropriate regulatory authorities. The oral route was selected as the most appropriate route of exposure and the results are believed to be of value in predicting the likely toxicity of the test item to man.

2.2 Justification for Test System

The rat is the preferred species as it has historically been used for safety evaluation studies and is the preferred species in the international test guidelines. The number of animals used was considered to be the minimum required to meet the scientific and regulatory objectives of the study.

3.0 MATERIALS AND METHODS

3.1 Test Item

Sponsor's identification	:	Paraquat SG (A19087K)
Description	:	Green granular solid
Batch number	:	J8813/180
Purity	:	Paraquat (ion): 49.4% w/w, corresponding to 494 g/kg PP796: 0.157% w/w, corresponding to 1.57 g/kg
Date received	:	20 April 2012
Expiry date	:	01 March 2015
Storage conditions	:	Room temperature in the dark

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

A Certificate of Analysis supplied by the Sponsor is given in Appendix 1.

For the purpose of the study the test item was freshly prepared, as required, as a solution at the appropriate concentration in distilled water.

The test item was formulated within two hours of being applied to the test system. It is assumed that the formulation was stable for this duration.

No analysis was conducted to determine the homogeneity, concentration or stability of the test item formulation. This is an exception with regard to GLP and has been reflected in the GLP compliance statement.

3.2 Experimental Design

3.2.1 Animals

Six female Wistar (RccHan™:WIST) strain rats were supplied by Harlan Laboratories UK Ltd., Oxon, UK.

On receipt the animals were randomly allocated to cages. The animals were nulliparous and non-pregnant. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study and identified by a number written on a cage card. At the start of the study the animals were eight to twelve weeks of age. The bodyweights fell within an interval of ± 20% of the mean weight of any previously dosed animals.

The animals were individually housed in suspended solid-floor polypropylene cages furnished with woodflakes. With the exception of an overnight fast immediately before dosing and for approximately three to four hours after dosing, free access to mains drinking water and food (2014C Teklad Global Rodent diet supplied by Harlan Laboratories U.K. Ltd., Oxon, UK) was allowed throughout the study. The diet, drinking water and bedding were routinely analysed and were considered not to contain any contaminants that would reasonably be expected to affect the purpose or integrity of the study.

The temperature and relative humidity were set to achieve limits of 19 to 25°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light (06:00 to 18:00) and twelve hours darkness.

The animals were provided with environmental enrichment items which were considered not to contain any contaminant of a level that might have affected the purpose or integrity of the study.

3.2.2 Procedure

No information was available regarding the toxicity of the test item therefore the default values for LD_{50} and sigma were entered into AOT425 Statistical Program. The statistical program gave a recommended dose progression of 5000, 1750, 550, 175, 55.0, 17.5, 5.5 and 1.75 mg/kg.

At the request of the Sponsor the first animal was dosed at 175 mg/kg. Further animals were then treated as follows based on the short-term results of the previously treated animal:

Test Sequence (Animal number)	Dose Level mg/kg	Concentration (mg/ml)	Dose Volume (ml/kg)	Short-Term Result
1 (1-0)	175	17.5	10	0
2 (2-0)	550	55	10	X
3 (3-0)	175	17.5	10	0
4 (4-0)	550	55	10	X
5 (5-0)	175	17.5	10	X
6 (6-0)	55	5.5	10	0

X = Animal died
0 = Animal survived

The test was complete after the sixth animal had been dosed as the following stopping criterion was met:

- at least four animals have followed the first reversal and the specified likelihood-ratios exceeded the critical value (calculations are made at each dosing, following the fourth animal after the first reversal)

All animals were dosed once only by gavage, using a metal cannula attached to a graduated syringe. The volume administered to each animal was calculated according to the fasted bodyweight at the time of dosing. Treatment of animals was sequential. Sufficient time (at least 48 hours) was allowed between each individual animal to confirm the outcome of the previously dosed animals.

The animals were observed for deaths or overt signs of toxicity ½, 1, 2 and 4 hours after dosing and subsequently once daily for up to fourteen days.

Individual bodyweights were recorded prior to dosing and seven and fourteen days after treatment or at death.

At the end of the observation period the surviving animals were killed by cervical dislocation. Animals were subjected to gross pathological examination. This consisted of an external examination and opening of the abdominal and thoracic cavities for examination of major organs. The appearance of any macroscopic abnormalities was recorded. No tissues were retained.

3.2.3 Evaluation of Data

The oral LD_{50} will be calculated by the maximum likelihood method. Data evaluations also included the relationship, if any, between the exposure of the animal to the test item and the incidence and severity of all abnormalities including behavioural and clinical observations, gross lesions, bodyweight changes, mortality and any other toxicological effects.

Using the mortality data obtained, an estimate of the acute oral median lethal dose (LD_{50}) of the test item was calculated by the statistical program.

4.0 RESULTS AND DISCUSSION

Individual mortality data and individual clinical observations are given in Table 1.

4.1 Mortality Data

The mortality data are summarised as follows:

Dose Level mg/kg	Number of Animals Survived	Number of Animals Died	Total Number of Animals
55	1	0	1
175	2	1	3
550	0	2	2
All doses	3	3	6

4.2 Clinical Observations

Signs of systemic toxicity noted were hunched posture, ataxia, pilo-erection and noisy respiration. There were no signs of systemic toxicity noted in one animal treated at a dose level of 175 mg/kg or in the animal treated at a dose level of 55 mg/kg.

4.3 Bodyweight

Individual bodyweights and weekly bodyweight changes are given in Table 2.

The surviving animals showed expected gains in bodyweight over the study period.

4.4 Necropsy

Individual necropsy findings are given in Table 3.

Abnormalities noted at necropsy of animals that died during the study were haemorrhagic lungs, dark liver, pale spleen, dark kidneys, haemorrhagic gastric mucosa and non-glandular epithelium of the stomach and dark green coloured material or green liquid present in the

stomach. No abnormalities were noted at necropsy of the remaining animals that were killed at the end of the study.

5.0 CONCLUSION

The acute oral median lethal dose (LD_{50}) of the test item in the female Wistar strain rat was calculated to be 175 mg/kg bodyweight (based on an assumed sigma of 0.5).

TABLES SECTION

GLOSSARY FOR TABLE 1

0	No signs of systemic toxicity
A	Ataxia
H	Hunched posture
Rn	Noisy respiration
P	Pilo-erection
X	Animal dead
-	No data, animal dead

TABLE 1 Individual Clinical Observations and Mortality Data

Dose Level mg/kg	Animal Number and Sex	Effects Noted After Dosing (Hours)				Effects Noted During Period After Dosing (Days)													
		½	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
175	1-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
550	2-0 Female	HA	HA	H	0	0	X												
175	3-0 Female	0	0	0	0	0	HRn	HRn	HRnP	H	0	0	0	0	0	0	0	0	0
550	4-0 Female	0	0	0	H	X													
175	5-0 Female	0	0	0	0	H	HP	X											
55	6-0 Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 2 Individual Bodyweights and Weekly Bodyweight Changes

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight (g) at Death	Bodyweight Gain (g) During Week	
		0	7	14		1	2
175	1-0 Female	153	155	182		2	27
550	2-0 Female	174	-	-	151	-	-
175	3-0 Female	180	185	204		5	19
550	4-0 Female	175	-	-	158	-	-
175	5-0 Female	165	-	-	145	-	-
55	6-0 Female	149	170	181		21	11

TABLE 3 Individual Necropsy Findings

Dose Level mg/kg	Animal Number and Sex	Time of Death	Macroscopic Observations
175	1-0 Female	Killed Day 14	No abnormalities detected
550	2-0 Female	Found dead Day 2	Lungs: haemorrhagic Liver: dark Spleen: pale Stomach: dark green material present Gastric mucosa: haemorrhagic Non-glandular epithelium of the stomach: haemorrhagic
175	3-0 Female	Killed Day 14	No abnormalities detected
550	4-0 Female	Found dead Day 1	Liver: dark Kidneys: dark Stomach: green liquid present
175	5-0 Female	Found dead Day 3	Liver: dark Kidneys: dark Stomach: epithelial sloughing
55	6-0 Female	Killed Day 14	No abnormalities detected

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

A19087K
R9910 as PP148 SG (50)
J8813/180

Batch Identification	**J8813/180**
Product Code	**A19087K**
Other Product Code(s)	R9910 as PP148 SG (50)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of paraquat (ion) ***	**49.4 % w/w corresponding to 494 g/kg**
– **Content of PP796 ***	**0.157 % w/w corresponding to 1.57 g/kg**

The Active Ingredient(s) content is within the FAO limits.
Methodology used for Characterization HPLC

Physical Analysis

– **Appearance**	**green solid**

Stability:

– **Storage Temperature**	**< 30°C**
– **Recertification Date**	**End of March 2015**

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: 124488
Study number(s) of batch recertification:

Authorisation:

18. MAI 2012

Dr. S. Adolph
Analytical Development & Product Chemistry

APPENDIX 2 Statement of GLP Compliance in Accordance with Directive 2004/9/EC

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY	TEST TYPE
Harlan Laboratories Ltd. **Shardlow Business Park** **London Road** **Shardlow** **Derbyshire** **DE72 2GD** ***This facility includes a field station at Crowle in Worcestershire***	**Analytical Chemistry** **Environmental Fate** **Environmental Toxicity** **Mutagenicity** **Phys/Chem Testing** **Residue Studies** **Toxicology**

DATE OF INSPECTION

19 – 21 July 2011

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK GLP Compliance Programme.

At the time of inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

31/8/11

Dr. Andrew J. Gray
Head, UK GLP Monitoring Authority

MHRA