Propiconazole/Fenpropidin

Propiconazole/Fenpropidin EC (A9050B) - Skin Sensitization in Guinea Pigs by the Buehler Method (9 Induction)

Final Report

DATA REQUIREMENT(S): OECD Test Guideline 406 (1992)
EPA OPPTS 870.2600 (2003)
Directive 440/2008/EC B.6 (2008)

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STUDY COMPLETION DATE: 05 May 2011

PERFORMING LABORATORY: LAB Research Ltd.
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Hungary

LABORATORY PROJECT ID: Report Number: 10/290-104T
Study Number: 10/290-104T
Task Number: TK0037096

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

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study has been performed in accordance with the study plan agreed upon by Sponsor, the OECD Guidelines for Testing of Chemicals No.: 406 (17 July 1992), US Environmental Protection Agency Health Effect Division Test Guideline OPPTS 870.2600 (March 2003), Commission Regulation (EC) No 440/2008 of 30 May 2008 and the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 9/2001. (III. 30.) EüM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development which corresponds to the OECD GLP, ENV/MC/CHEM (98)17).

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: Bme Török Magdolna Date: 05 May 2011

Magdolna Török-Batho, M.Sc.
Study Director

Performing Laboratory: LAB Research Ltd.
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FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Code: 10/290-104T

Study Title: Propiconazole/Fenpropidin EC (A9050B) - Skin Sensitization in Guinea Pigs by the Buehler Method (9 Induction)

Test Item: Propiconazole/Fenpropidin EC (A9050B)

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 are given below:

Date of Inspection	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
18 October 2010	Study Plan	18 October 2010	18 October 2010
14 February 2011	Treatment	14 February 2011	14 February 2011
12 April 2011	Draft Report	12 April 2011	12 April 2011
05 May 2011	Final Report	05 May 2011	05 May 2011

Signature: Heiderné Grób Ramóna Date: 05 May 2011

Ramóna Grób Heiderné, B.Sc.
On behalf of QA

MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd. (as Sponsor) and LAB Research Ltd. (as Testing Facility), the study titled "Propiconazole/Fenpropidin EC (A9050B) - Skin Sensitization in Guinea Pigs by the Buehler Method (9 Induction)" was performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 05 May 2011

Christopher Banks, DABT
Managing Director

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

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Study dates

Experimental I. Starting Date:	07 November 2010
Termination:	12 November 2010
Experimental II. Starting Date:	06 February 2011
Experimental Completion Date	13 March 2011
Treatment (main study):	07 February to 11 March 2011
Observation (main study):	07 February to 13 March 2011
Termination:	13 March 2011

Deviation to the guidelines

Concerning: Animal husbandry
According to the Study Plan: Humidity will be 30-70%
Deviation: Humidity was between 10 – 42% during the main test
Reason: Technical reason

Concerning: Animal husbandry
According to the Study Plan: Temperature will be 20 ± 3°C
Deviation: Temperature was between 19.4 – 25.0 °C during the main test
Reason: Technical reason

These deviations have no affect on the results or integrity of the study.

Performing laboratory test substance reference number

10/238K/1 101004

Other

The study documents:
- study plan and amendments,
- all raw data,
- sample of the test item,
- study report and any amendment,
- correspondence

will be stored in the archives of LAB Research Ltd., 8200 Veszprém-Szabadságpuszta, Hungary according to the Hungarian GLP regulation and to test facility SOPs.
After the retention time agreed with the Sponsor has elapsed, all the archived materials listed above will be offered to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded. No raw data or material relating to the study will be discarded without the Sponsor's prior written consent.

TABLE OF CONTENTS

STATEMENT OF DATA CONFIDENTIALITY CLAIMS		**2**
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT		**3**
FLAGGING STATEMENT		**4**
QUALITY ASSURANCE STATEMENT		**5**
MANAGEMENT STATEMENT		**6**
GENERAL INFORMATION		**7**
TABLE OF CONTENTS		**9**
1.0	**EXECUTIVE SUMMARY**	**11**
1.1	Study Design	11
1.2	Results	11
1.3	Conclusion	11
2.0	**INTRODUCTION**	**12**
2.1	Purpose	12
2.2	Guidelines	12
2.3	Sensitivity of the Test System	12
2.4	Test Facility	12
3.0	**MATERIALS AND METHODS**	**12**
3.1	Test Item	12
3.1.1	Identification, receipt	13
3.1.2	Formulation	13
3.2	Vehicle	13
3.3	Subsidiary Materials	14
3.4	Experimental Design	14
3.4.1	Animals	14
3.4.2	Husbandry	15
3.4.3	Food and feeding	15
3.4.4	Water supply	15
3.4.5	Identification	16
3.4.6	Randomization	16
3.4.7	Measurement of body weight	16
3.5	Test Procedure	16
3.5.1	Preliminary dose range finding study	16
3.5.2	Main study: dermal induction	18
3.5.3	Main study: challenge exposure (Day 33)	18

3.6	Observation and Scoring	18
3.7	Evaluation	19
3.8	Positive Control	19
4.0	**RESULTS AND DISCUSSION**	**21**
4.1	Main Study	21
4.1.1	Test group	21
4.1.2	Control group	21
4.2	Body weight	21
5.0	**CONCLUSIONS**	**21**
6.0	**REFERENCES**	**21**
TABLES SECTION		**22**
TABLE 1	Preliminary Dose Range Finding Study Observations	23
TABLE 1	Preliminary Dose Range Finding Study Observations (Continued)	24
TABLE 1	Preliminary Dose Range Finding Study Observations (Continued)	25
TABLE 1	Preliminary Dose Range Finding Study Observations (Continued)	26
TABLE 2	Skin Reactions Observed during Induction	27
TABLE 3	Dermal Response Scores for Guinea Pigs Challenged with Test Item (Control and Test Group)	28
TABLE 4	Individual Bodyweight Data	30
TABLE 5	Dermal Response Scores for Guinea Pigs Challenged with Reference Item 2-Mercaptobenzothiazole (Control and Test Group)	31
APPENDICES SECTION		**32**
APPENDIX 1	Certificate of Analysis	33
APPENDIX 2	GLP Certificate	34

1.0 EXECUTIVE SUMMARY

1.1 Study Design

The purpose of this skin sensitization study was to assess the possible allergenic potential of Propiconazole/Fenpropidin EC (A9050B) when administered topically to albino Guinea pigs.

For this purpose, the Buehler Method was used [1]. Twenty male animals of the test group were treated topically with 10 (w/v) % Propiconazole/Fenpropidin EC (A9050B) in distilled water on 9 occasions during the induction phase. Fourteen days after the final induction application, the animals were challenged with a single application of the test item at concentrations of 10 and 5 (w/v) % formulation in distilled water.

Ten animals of the control group were treated with distilled water during the induction period. The control animals were challenged with the test item at concentrations of 10 and 5 (w/v) % in distilled water.

1.2 Results

No signs of systemic toxicity were observed in any animal.

During the induction, very slight to well defined erthyma (scores 1-2) was observed in test animals. Additionally, dry, scaly and cracked skin were recorded in all animals at the end of the induction phase. No local skin effects were observed in the control group animals.

Following challenge with 10 and 5 (w/v) % Propiconazole/Fenpropidin EC (A9050B) in distilled water, there were no skin reactions in the test or control animals.

1.3 Conclusion

Based on the results of this study, the test item Propiconazole/Fenpropidin EC (A9050B) is considered not to be a skin sensitizer in the Guinea pig.

2.0 INTRODUCTION

2.1 Purpose

The purpose of this skin sensitization study was to assess the possible allergenic potential of Propiconazole/Fenpropidin EC (A9050B) when administered topically to albino guinea pigs.

2.2 Guidelines

The study was performed according to the following guidelines:

OECD Guidelines for Testing of Chemicals, Number 406 "Skin Sensitization", adopted by the Council on July 17, 1992 (reported Paris, April 29, 1993).

United States Environmental Protection Agency, Health Effects Division Test Guidelines, OPPTS 870.2600 Skin Sensitization EPA 712-C-03-197, March 2003.

Directive 440/2008/EC B.6 (2008) of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), B.6. Skin sensitisation (Official Journal No L 142, 31/05/2008 p. 0202-0209).

2.3 Sensitivity of the Test System

The sensitivity and reliability of the experimental technique employed was assessed by use of 2-Mercaptobenzothiazole (CAS number: 149-30-4) , which are recommended by the OECD 406 Guidelines and are known to have moderate skin sensitization properties in the guinea pig strain. The results from the most recent test (LAB Research Ltd. study number 11/020-104T), was performed from 21 February 2011 to 24 March 2011 are included in the section 3.8.

2.4 Test Facility

The conduct of the study was approved by the Animal Care and Use Committee (IACUC) of LAB Research Ltd.

3.0 MATERIALS AND METHODS

3.1 Test Item

Name:	Propiconazole / Fenpropidin EC (A9050B)
Batch number:	SMO0G120
Purity:	Propiconazole – 129 g/L corresponding to 13.6 % w/w Fenpropidin – 278 g/L corresponding to 29.2 % w/w
Product code:	A9050B

Other Product Code:	CGA64250/CGA114900 EC (127/275)
Appearance:	Brown liquid
Density:	952 kg/m^3
Reanalysis date:	End of September 2015
Storage conditions:	Room temperature (<30^{o}C)
Safety Precautions:	Routine safety precautions (lab coat, gloves, goggles, face mask) for unknown materials were applied to assure personnel health and safety.

Certificate of Analysis is presented in Appendix 1.

3.1.1 Identification, receipt

The test item of a suitable chemical purity was supplied by the Sponsor. All precautions required in the handling and disposal of the test item were outlined by the Sponsor. The identification of test item was made using its name, batch number, appearance and color in the Central Dispensary Unit of LAB Research Ltd.

3.1.2 Formulation

Before the preliminary dose range finding study, a preliminary test item formulation evaluation was performed. The solubility of the test item was examined in distilled water. The use of distilled water resulted in a formulation suitable for dosing, therefore it was used as the vehicle (according to the principles of the relevant guidelines).

On the basis of the results of the test item formulation evaluation and the preliminary dose range finding study, the test item was used in the main study at the following concentrations:

- Induction exposures - 10 (w/v) %
- Challenge exposure - concentrations of 10 (w/v) % in distilled water on the left flank and 5 (w/v) % in distilled water on the right flank.

The test item was weighed and formulations were prepared daily on a weight:volume basis (as (w/v) %) in the Central Dispensary Unit of LAB Research Ltd.

3.2 Vehicle

Name:	Distilled water
Batch No.:	7530810
Manufacturer:	TEVA Co.
Expiry:	August 2013
Storage condition:	Room temperature

3.3 Subsidiary Materials

Name:	Acidum ascorbicum
Batch No.:	DY0261030501
Expiry:	22 July 2013
Name:	Sterile gauze patch
Batch/lot number:	100317142 ; 90 15 43392
Expiry date:	March 2015 ; December 2014
Name:	Silkplast
Lot No.:	376/24 ; 693/25
Expiry Date:	June 2013 ; December 2014
Name:	Omnifix ® Tuberculin Syringe (1 mL)
Lot No.:	0A18048
Expiry Date:	January 2015
Name:	Euthasol® (40%)
Batch No.:	10C25 7
Expiry:	February 2013
Produced by:	AST Beheer B.V., Oudewater, Netherlands (Produlab Pharma, Raamsdonksveer)
Storage condition:	Room temperature

3.4 Experimental Design

3.4.1 Animals

Species and strain:	Guinea pigs, LAL/HA/BR
Source:	LAB-ÁLL Bt. Budapest, 1174 Hunyadi u. 7.
Justification of strain:	The guinea pig is the standard species used for skin sensitization studies.
Number of animals	
Range finding study:	13 animals
Main study:	
Test group:	20 animals
Control group:	10 animals
Sex:	Male
Age of animals at arrival:	Young adult
Weight range at the beginning of the study:	348 - 428 g (The weight variation in animals involved in the study did not exceed ± 20% of the mean weight)
Acclimatization time:	12 days

3.4.2 Husbandry

Animal health:	Only animals in acceptable health condition were used for the test. Health status was certified by the veterinarian.
Room/Cabinet:	602/5-6
Housing/Enrichment:	Animals were housed in macrolon cages size III (42 x 42 x 19 cm), with 2 or 3 animals/cage to allow socialization
Bedding:	Laboratory bedding, Lignocel 3-4 Fasern (produced by J. Rettenmaier & Söhne GmbH+CO.KG, D-73494 Rosenberg, Germany) was available to animals during the study.
Temperature during the main study:	19.4 – 25.0 °C
Relative humidity: during the main study	10 – 42%
Ventilation:	15-20 air exchanges/hour

The environmental parameters were recorded twice daily during the study. Variations from the target humidity range were observed during the study. These deviations were considered to have no impact on the animal health, as certified by the Clinical Veterinarian, or on the outcome of the study and interpretation of the results due to their low magnitude.

3.4.3 Food and feeding

The animals received Purina Base – Lap gr. diet for rabbit produced by Agribrands Europe Hungary PLC, H-5300 Karcag, Madarasi road, Hungary, *ad libitum*. This diet is classified as being suitable for Guinea pigs as the vitamin D level is high enough to meet the needs of this species. This is the diet used by the breeder/supplier, therefore animals are fully adapted to this diet on arrival. The details of the diet or diets used will be archived with the raw data and not reported.

3.4.4 Water supply

Animals received tap water from municipal supply as for human consumption, with the addition of 50 mg/100 mL ascorbic acid, *ad libitum*. The drinking water is routinely analysed and is considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

The 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.u.36., Hungary). Copies of the relevant Certificates of Analysis are retained in the archives at LAB Research Ltd.

3.4.5 Identification

The animals were marked individually with ear punching. The cages were marked with individual identity cards with information about study code, sex, cage number, dose group and individual animal number.

3.4.6 Randomization

On the day prior to treatment, all animals were weighed and randomly allocated to treatment group based on body weight. The randomization was checked to ensure homogeneity of means and standard deviations between groups.

3.4.7 Measurement of body weight

The body weights of individual animals were recorded at the beginning and at the end of the experiment. The mean values and the standard deviations were calculated in the control and test groups.

3.5 Test Procedure

3.5.1 Preliminary dose range finding study

Start of preliminary study: 19 October 2010
End of preliminary study: 25 January 2011

The vehicle was selected on the basis of solubility of the test substance and in accordance with OECD guideline 406. The maximum achievable concentration was greater than 75 (w/v) % in distilled water. Four dose levels were tested in the first step of preliminary dose range finding study to identify any primary irritation by dermal application. Approximately 24 hours prior to the test, the hair was removed from the right and left surface of the animals (approximately 5 x 5 cm). The hair removal was performed carefully to ensure it was closely shaven.

For the dermal application a 2.5 x 2.5 cm gauze patch was fully loaded with approximately 0.5 mL formulated test item was applied to a 5 x 5 cm gauze patch and the patch placed onto the skin of the animals at concentrations of 100% (undiluted), 75, 50 and 25 (w/v) %. A closed patch exposure was employed by means of an occlusive bandage. Two animals were used to test the dermal concentrations. One concentration was used on the right side and another concentration on left side of animal.

It was found that 0.5 mL of test formulations at concentrations of 100% (undiluted), 75, 50 and 25 (w/v) % formulations produced no reaction (scores 0-0) on the skin of guinea pigs.

On the basis of the results of the preliminary test item formulation evaluation and the Preliminary Dose Range Finding Study, the test item was used for the dermal induction

treatment at a concentration of 100% (undiluted). Control animals were not treated although the dressing was applied.

Due to unexpected systemic toxicity following multiple applications of the test item in Main Experiment I., all the treated group Guinea pigs were terminated, with a minimal gross necropsy. The results of Main Experiment I. are not reported, however all the raw data are filed and archived in the study binder.

An additional Prelimianry Dose Range Finding Study was required to find the highest acceptable dose that was suitable for the main experiment. Three animals per dose group were treated in a stepwise manner with varying dilutions of the test item, mimicking the induction period of a Buehler study (up to 9 inductions). The animals were treated on one flank 3 times a week (Monday, Wednesday and Friday) for 3 weeks as described in 3.5.2 (or until the group was stopped due to toxicity). During the preliminary test all animals were observed at least once daily for any clinical signs, including systemic toxicity. Skin reactions were observed and recorded 24 hours after patch removal. Following the observation of excessive clinical signs at 100% (undiluted) test item, animals were treated at 50 (w/v) %, 25 (w/v) % and 10 (w/v) % in distilled water.

Treatment with 50 (w/v) % test item resulted in a slight body weight loss in all 3 animals. Following 24 hours after the fifth induction treatment, the degree of irritation (including skin cracking and scaly, indicating loss of skin integrity) was greater than would be acceptable on a main study. Therefore the animals were terminated for animal welfare and scientific reasons.

A further 3 animals were treated with 10 (w/v) % test item for up to 3 weeks. Minor clinical symptoms (dry and cracked skin) were observed and there was no significant effect on body weight. 10 (w/v) % test item was considered well tolerated and acceptable for use on the main study.

A further 3 animals were treated with 25 (w/v) % test item for up to 3 weeks to ensure that the remaining dose range had been explored. Before the 6th treatment, the severity of the irritation increased such that the degree of irritation (including skin cracking, indicating loss of skin integrity) was greater than would be acceptable on a main study.

On the basis of the results of the additional Preliminary Dose Range Finding Study 10 (w/v) % of the test item in distilled water was considered the most appropriate dose and was used for the dermal induction treatment in the main experiment. Control animals were treated with distilled water during the induction period.

For the challenge exposure, all animals of the treatment and control group were treated with a 10 (w/v) % concentration on the left flank of each animal. The right shaved flank area of all animals were treated with 5 (w/v) % test item in distilled water.

All Preliminary Dose Range Finding Study results observed are summarized in Table 1.

3.5.2 Main study: dermal induction

Approximately 24 hours before the first treatment, an area approximately 5 x 5 cm on the back region of all animals was carefully shaved. The test animals were treated with approximately 0.5 mL of 10 (w/v) % formulation of the test item. A 5 x 5 cm patch of sterile gauze patch was applied. An approximately 2.5 x 2.5 cm area of this gauze patch was saturated with 10 (w/v) % and placed on the skin of the animals. These gauze patches were kept in contact with the skin by a patch with a surrounding adhesive hypoallergenic plaster (closed patch test). The control group animals were treated with distilled water.

Each animal was treated 3 times per week (Monday, Wednesday and Friday) for 3 weeks. If hair growth became excessive, the exposed surface was shaved 1 day (or at least 1 hour) before the next treatment (the procedure was recorded on the data sheet).

The duration of induction exposure was 6 hours. After the patch removal, any remaining test item was removed with a gauze swab and warm water.

3.5.3 Main study: challenge exposure (Day 33)

Two weeks after the last dermal induction treatment, the animals were exposed to challenge doses. All animals of the treatment and control groups were treated with a concentration of 10 (w/v) % formulations on the left shaved flank. The right shaved flank area of all animals was treated with 5 (w/v) % test item in distilled water. Dosing was performed as described in Section 3.5.2 (closed patch test). The volume of the formulated test item was approximately 0.5 mL and the time of exposure was 6 hours.

3.6 Observation and Scoring

For the range-finding study (primary irritation) and readings during the induction phase of the main study, the dermal irritation scores (were evaluated according to the scoring system by Draize (1959) presented in the following table.

Erythema and eschar formation

No Erythema	0
Very slight Erythema (barely perceptible)	1
Well defined Erythema	2
Moderate to severe Erythema	3
Severe Erythema (beef redness) to slight eschar formation (injuries in depth)	4

Oedema formation

No odema	0
Very slight oedema (barely perceptible)	1

Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised appr. 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

CLASSIFICATION OF SKIN IRRITATION

0	=	non irritant
1	=	slightly irritant
2-3	=	mildly to moderately irritant
4	=	severely irritant

The dermal induction treatments were observed and recorded (See Table 2). Records are archived with the raw data.

3.7 Evaluation

After the challenge exposure, each animal was examined and scored. Grading was performed 24 and 48 hours after the end of the exposure. Scoring was performed according to the following system:

0	=	no visible change
1	=	discrete or patchy erythema
2	=	moderate and confluent erythema
3	=	intense erythema and swelling

To classify the sensitization responses, the primary irritation of the test item has to be taken into consideration. The percentage of animals showing a positive reaction was calculated in both test and common challenge control groups. As a result of these, the percentage value of the control animals that responded were subtracted from the percentage of the test animals responded positively to the challenge. The net response value resulted in the sensitization rate in percent.

According to the Buehler procedure, if at least 15% of the animals show an allergic response, after the challenge, the test item is classified as a "sensitizer".

3.8 Positive Control

The sensitivity and reliability of the experimental procedure is assessed twice a year by use of 2-Mercaptobenzothiazole (CAS number: 149-30-4) which is known to have moderate skin sensitization properties (OECD 406, adopted 17 July 1992 chapter 10., 11.).

The results of the latest reliability check (Study code: 11/020-104T):

Start of Experiment: 21 February 2011
End of Experiment: 24 March 2011

Reference Items and Vehicle

Test Item:	2-Mercaptobenzothiazole
Lot number:	MKBB4881
CAS Number:	149-30-4
Expiry date:	08 February 2012
Manufacturer:	Sigma-Aldrich Co.
Storage condition:	Room temperature

Component of vehicle:

Name:	Methylcellulosum
Synonym:	Methylcellulose
Batch number:	K93935287
Manufacturer:	Dow Chemicals
Expiry Date:	May 2012
Storage condition:	Room temperature, protected from light

Name:	Humaqua (Aqua Destillata pro Injectione)
Batch No.:	7530810
Manufacturer:	TEVA Co.
Expiry:	August 2013
Storage condition:	Room temperature

SUMMARY OF THE RELIABILITY STUDY

Challenge with the reference item elicited discrete erythema on the skin surface of previously sensitised guinea pigs. The mean of the scores were 0.20 (20% of animals) and 0.20 (20% of animals) at the 24 and 48 hour observations. The dermal scores observed on the skin surface of previously sensitized guinea pigs represented discrete erythema. On the opposite (right) side was treated with vehicle only, no reaction was found.

In the concurrent control group, the mean of the scores was 0.00.

The results are summarized in Table 5.

According to the net percentage value of positively responded animals and to the net score value of the skin reactions, the test item 2-Mercaptobenzothiazole was classified as a sensitiser. This demonstrated that the experimental procedure was successful.

4.0 RESULTS AND DISCUSSION

4.1 Main Study

4.1.1 Test group

No signs of systemic toxicity were observed in any animal.

During the induction, very slight to well defined erthyma (scores 1-2) was observed in test animals. Additionally, dry, scaly and cracked skin were recorded in all animals at the end of the induction phase.

The observations are summarised in Table 2.

Following challenge with 10 and 5 (w/v) % Propiconazole/Fenpropidin EC (A9050B) in distilled water, there were no skin reactions in the test animals (Table 3).

4.1.2 Control group

No skin reactions were observed in any control animals during the induction phase (Table 2).

Challenge observations are summarized in Table 3. No positive responses were observed in the control group, the mean of the score was 0.00 at the 24 and 48 hour observation following a challenge with 10 and 5 (w/v) % Propiconazole/Fenpropidin EC (A9050B) in distilled water.

4.2 Body weight

Normal body weight gain was noted for all animals. There were no differences between the control and treated groups. The mean values of the groups are summarized in Table 4.

5.0 CONCLUSIONS

Based on the results of this study, the test item Propiconazole/Fenpropidin EC (A9050B) is considered not to be a skin sensitizer in the guinea pig.

6.0 REFERENCES

1. Buehler, E. V(1965). Delayed contact hypersensitivity in the guinea pig. Arc. Dermatol. 91: 171-177.
2. Draize JH (1959). Dermal toxicity. Appraisal of the safety of chemicals in foods, drugs and cosmetics. Association of food and drug officials of the United States, Texas State Department of Health. Texas: Austin.
3. EEC Directive 2001/59/EC, (6 August 2001)

TABLES SECTION

TABLE 1 Preliminary Dose Range Finding Study Observations

Test item: Propiconazole/Fenpropidin EC (A9050B)
Vehicle: Distilled water

Propiconazole/ Fenpropidin EC (A9050B)	ANIMAL NUMBER	SKIN IRRITATION			
		1 hour	24 hours	48 hours	72 hours
		after the patch removal			
100 % (Undiluted) (dermally)	38	0E, 0O	0E, 0O	0E, 0O	0E, 0O
	47	0E, 0O	0E, 0O	0E, 0O	0E, 0O
75 (w/v) % (dermally)	38	0E, 0O	0E, 0O	0E, 0O	0E, 0O
	47	0E, 0O	0E, 0O	0E, 0O	0E, 0O
50 (w/v) % (dermally)	55	0E, 0O	0E, 0O	0E, 0O	0E, 0O
	56	0E, 0O	0E, 0O	0E, 0O	0E, 0O
25 (w/v) % (dermally)	55	0E, 0O	0E, 0O	0E, 0O	0E, 0O
	56	0E, 0O	0E, 0O	0E, 0O	0E, 0O

Scoring and Assessment of Local Reactions

Erythema and Eschar Formation Oedema Formation

No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well defined erythema	2	Slight oedema (edges of area well defined by definite raising)	2
Moderate to severe erythema	3	Moderate oedema (raised appr. 1 mm)	3
Severe erythema (beef redness) to slight eschar formation (injures in depth)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Abbreviations: E = Erythema O = Oedema

TABLE 1 Preliminary Dose Range Finding Study Observations (Continued)

Propiconazole/ Fenpropidin EC (A9050B)	ANIMAL NUMBER	LOCAL and SYSTEMIC OBSERVATIONS				
		24 hours after the 1st treatment	24 hours after the 2nd treatment	24 hours after the 3rd treatment	24 hours after the 4th treatment	24 hours after the 5th treatment
50 (w/v) % (dermally)	92	1E, 0O No Systemic signs	1E*, 0O No Systemic signs	1E*, 0O No Systemic signs	2E*, 0O No Systemic signs	2E*, 0O, T No Systemic signs
50 (w/v) % (dermally)	94	1E, 0O No Systemic signs	1E*, 0O No Systemic signs	1E*, 0O No Systemic signs	2E*, 0O No Systemic signs	2E*, 0O, T No Systemic signs
50 (w/v) % (dermally)	96	1E, 0O No Systemic signs	1E*, 0O No Systemic signs	1E*, 0O No Systemic signs	2E*, 0O No Systemic signs	2E*, 0O, T No Systemic signs

Scoring and Assessment of Local Reactions

Erythema and Eschar Formation		**Oedema Formation**	
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well defined erythema	2	Slight oedema (edges of area well defined by definite raising)	2
Moderate to severe erythema	3	Moderate oedema (raised appr. 1 mm)	3
Severe erythema (beef redness) to slight eschar formation (injures in depth)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Abbreviations: E = Erythema O = Oedema

*: dry, scaly and cracked skin
T: Terminated due to excessive skin irritation

TABLE 1 Preliminary Dose Range Finding Study Observations (Continued)

Propiconazole/ Fenpropidin EC (A9050B)	ANIMAL NUMBER	LOCAL and SYSTEMIC OBSERVATIONS		
		24 hours after the 1st treatment	24 hours after the 2nd treatment	24 hours after the 3rd treatment
10 (w/v) % (dermally)	98	0E, 0O No Systemic signs	0E, 0O No Systemic signs	0E*, 0O No Systemic signs
10 (w/v) % (dermally)	104	0E, 0O No Systemic signs	0E, 0O No Systemic signs	0E*, 0O No Systemic signs
10 (w/v) % (dermally)	105	0E, 0O No Systemic signs	0E, 0O No Systemic signs	0E*, 0O No Systemic signs
Propiconazole/ Fenpropidin EC (A9050B)	ANIMAL NUMBER	LOCAL and SYSTEMIC OBSERVATIONS		
		24 hours after the 4st treatment	24 hours after the 5th treatment	24 hours after the 6th treatment
10 (w/v) % (dermally)	98	0E*, 0O No Systemic signs	0E*, 0O No Systemic signs	0E*, 0O No Systemic signs
10 (w/v) % (dermally)	104	0E*, 0O No Systemic signs	0E*, 0O No Systemic signs	0E*, 0O No Systemic signs
10 (w/v) % (dermally)	105	0E*, 0O No Systemic signs	0E*, 0O No Systemic signs	0E*, 0O No Systemic signs
Propiconazole/ Fenpropidin EC (A9050B)	ANIMAL NUMBER	LOCAL and SYSTEMIC OBSERVATIONS		
		24 hours after the 7th treatment	24 hours after the 8th treatment	24 hours after the 9th treatment
10 (w/v) % (dermally)	98	0E**, 0O No Systemic signs	0E**, 0O No Systemic signs	0E**, 0O No Systemic signs
10 (w/v) % (dermally)	104	1E**, 0O No Systemic signs	0E**, 0O No Systemic signs	1E**, 0O No Systemic signs
10 (w/v) % (dermally)	105	0E**, 0O No Systemic signs	0E**, 0O No Systemic signs	0E**, 0O No Systemic signs

Scoring and Assessment of Local Reactions

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to slight eschar formation (injures in depth)	4

Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised appr. 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Abbreviations: E = Erythema
* = dry skin; ** = dry, cracked skin
O = Oedema

TABLE 1 Preliminary Dose Range Finding Study Observations (Continued)

Propiconazole/ Fenpropidin EC (A9050B)	ANIMAL NUMBER	LOCAL and SYSTEMIC OBSERVATIONS		
		24 hours after the 1st treatment	24 hours after the 2nd treatment	24 hours after the 3rd treatment
25 (w/v) % (dermally)	77	0E, 0O No Systemic signs	0E, 0O No Systemic signs	1E*, 0O No Systemic signs
25 (w/v) % (dermally)	78	0E, 0O No Systemic signs	0E, 0O No Systemic signs	1E*, 0O No Systemic signs
25 (w/v) % (dermally)	80	0E, 0O No Systemic signs	0E, 0O No Systemic signs	1E*, 0O No Systemic signs
Propiconazole/ Fenpropidin EC (A9050B)	ANIMAL NUMBER	LOCAL and SYSTEMIC OBSERVATIONS		
		24 hours after the 4st treatment	24 hours after the 5th treatment	24 hours after the 6th treatment
25 (w/v) % (dermally)	77	1E*, 0O No Systemic signs	1E*, 1O No Systemic signs	1E*, 1O No Systemic signs
25 (w/v) % (dermally)	78	1E*, 0O No Systemic signs	1E*, 1O No Systemic signs	1E*, 1O No Systemic signs
25 (w/v) % (dermally)	80	1E*, 0O No Systemic signs	1E*, 1O No Systemic signs	1E*, 1O No Systemic signs
Propiconazole/ Fenpropidin EC (A9050B)	ANIMAL NUMBER	LOCAL and SYSTEMIC OBSERVATIONS		
		24 hours after the 7st treatment	24 hours after the 8th treatment	24 hours after the 9th treatment
25 (w/v) % (dermally)	77	1E**, 1O, T No Systemic signs	-	-
25 (w/v) % (dermally)	78	1E**, 1O, T No Systemic signs	-	-
25 (w/v) % (dermally)	80	1E**, 1O, T No Systemic signs	-	-

Scoring and Assessment of Local Reactions

Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to slight eschar formation (injures in depth)	4

Oedema Formation

No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised appr. 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

Abbreviations: E = Erythema O = Oedema

* = dry and cracked skin; ** = dry, scaly and cracked skin
T = terminated due to excessive skin irritation; - = no data

TABLE 2 Skin Reactions Observed during Induction

Test item: Propiconazole/Fenpropidin EC (A9050B)

Test item concentration: 10 (w/v) % formulation in distilled water

Control Group

Animal number Male	1	5	9	16	18	20	21	22	25	27
Test day 2	0E	0E	0E	0E	0E	0E	0E	0E	0E	0E
Test day 4	0E	0E	0E	0E	0E	0E	0E	0E	0E	0E
Test day 6	0E	0E	0E	0E	0E	0E	0E	0E	0E	0E
Test day 9	0E	0E	0E	0E	0E	0E	0E	0E	0E	0E
Test day 11	0E	0E	0E	0E	0E	0E	0E	0E	0E	0E
Test day 13	0E	0E	0E	0E	0E	0E	0E	0E	0E	0E
Test day 16	0E	0E	0E	0E	0E	0E	0E	0E	0E	0E
Test day 18	0E	0E	0E	0E	0E	0E	0E	0E	0E	0E
Test day 20	0E	0E	0E	0E	0E	0E	0E	0E	0E	0E

Test Group

Animal number Male	2	3	4	6	7	8	10	12	13	14
Test day 2	0E	0E	0E	0E	0E	0E	0E	1E	0E	0E
Test day 4	0E*	0E	0E	0E*	0E	0E	0E	1E*	1E*	0E
Test day 6	0E	0E*	0E	0E	0E	0E	0E	1E*	1E*	1E*
Test day 9	1E*	0E*	0E*	0E*	0E*	0E*	0E*	0E*	0E*	0E*
Test day 11	0E*	0E*	0E*	0E*	0E*	0E*	0E*	1E*	0E*	0E*
Test day 13	1E*	1E*	1E*	1E*	1E*	1E*	1E*	1E*	1E*	1E*
Test day 16	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**
Test day 18	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**
Test day 20	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**

Test Group

Animal number Male	15	17	23	24	26	28	29	30	32	33
Test day 2	0E	1E	0E	0E	0E	0E	0E	0E	0E	1E
Test day 4	0E*	1E*	0E	0E*	0E	0E	0E	0E	0E	1E*
Test day 6	0E	1E*	0E	1E	0E	0E	0E	0E	0E	0E*
Test day 9	0E*	0E*	0E*	0E*	0E*	0E*	0E*	0E*	0E*	0E*
Test day 11	0E*	0E*	0E*	1E*	0E*	0E*	0E*	0E*	0E*	0E*
Test day 13	1E*	1E*	1E*	1E*	1E*	1E*	1E*	1E*	1E*	1E*
Test day 16	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**
Test day 18	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**
Test day 20	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**	2E**

0E = No erythema
1E = Very slight Erythema (barely perceptible)
2E = Well defined Erythema
*: dry skin; **: dry, scaly, cracked skin.

TABLE 3 Dermal Response Scores for Guinea Pigs Challenged with Test Item (Control and Test Group)

Test item: Propiconazole/Fenpropidin EC (A9050B)

Test item concentration: 10 (w/v) % formulation – left side

Test Animals			Control Animals		
ANIMAL NUMBER	SCORES OF DERMAL REACTION		ANIMAL NUMBER	SCORES OF DERMAL REACTION	
	24 hours after the patch removal	48 hours after the patch removal		24 hours after the patch removal	48 hours after the patch removal
2	0	0	1	0	0
3	0	0	5	0	0
4	0	0	9	0	0
6	0	0	16	0	0
7	0	0	18	0	0
8	0	0	20	0	0
10	0	0	21	0	0
12	0	0	22	0	0
13	0	0	25	0	0
14	0	0	27	0	0
15	0	0	-	-	-
17	0	0	-	-	-
23	0	0	-	-	-
24	0	0	-	-	-
26	0	0	-	-	-
28	0	0	-	-	-
29	0	0	-	-	-
30	0	0	-	-	-
32	0	0	-	-	-
33	0	0	-	-	-
mean of scores	0.00	0.00	mean of scores	0.00	0.00
number of positive/ number of tested	0/20	0/20	number of positive/ number of tested	0/10	0/10

TABLE 3 Dermal Response Scores for Guinea Pigs Challenged with Test Item (Control and Test Group) (Continued)

Test item: Propiconazole/Fenpropidin EC (A9050B)

Test item concentration: 5 (w/v) % formulation – right side

Test Animals			Control Animals		
ANIMAL NUMBER	SCORES OF DERMAL REACTION		ANIMAL NUMBER	SCORES OF DERMAL REACTION	
	24 hours after the patch removal	48 hours after the patch removal		24 hours after the patch removal	48 hours after the patch removal
2	0	0	1	0	0
3	0	0	5	0	0
4	0	0	9	0	0
6	0	0	16	0	0
7	0	0	18	0	0
8	0	0	20	0	0
10	0	0	21	0	0
12	0	0	22	0	0
13	0	0	25	0	0
14	0	0	27	0	0
15	0	0	-	-	-
17	0	0	-	-	-
23	0	0	-	-	-
24	0	0	-	-	-
26	0	0	-	-	-
28	0	0	-	-	-
29	0	0	-	-	-
30	0	0	-	-	-
32	0	0	-	-	-
33	0	0	-	-	-
mean of scores	0.00	0.00	mean of scores	0.00	0.00
number of positive/ number of tested	0/20	0/20	number of positive/ number of tested	0/10	0/10

TABLE 4 Individual Bodyweight Data

TEST GROUP		BODY WEIGHT (g)		CONTROL GROUP		BODY WEIGHT (g)	
ANIMAL NUMBER	SEX	Day 0	Day 35	ANIMAL NUMBER	SEX	Day 0	Day 35
2	Male	376	471	1	Male	409	498
3	Male	362	456	5	Male	392	476
4	Male	370	459	9	Male	348	432
6	Male	381	476	16	Male	392	459
7	Male	366	450	18	Male	428	511
8	Male	366	470	20	Male	419	500
10	Male	403	488	21	Male	366	447
12	Male	417	490	22	Male	377	453
13	Male	408	483	25	Male	367	464
14	Male	427	491	27	Male	376	435
15	Male	426	417	-	-	-	-
17	Male	410	492	-	-	-	-
23	Male	400	485	-	-	-	-
24	Male	381	480	-	-	-	-
26	Male	348	437	-	-	-	-
28	Male	371	466	-	-	-	-
29	Male	390	492	-	-	-	-
30	Male	399	500	-	-	-	-
32	Male	374	471	-	-	-	-
33	Male	380	478	-	-	-	-
mean value: ±SD N: 20 / 20	-	387.75 22.41	472.60 20.68	mean value: ±SD N: 10/10	-	387.40 25.43	467.50 27.85

TABLE 5 Dermal Response Scores for Guinea Pigs Challenged with Reference Item 2-Mercaptobenzothiazole (Control and Test Group)

Test item: 2-Mercaptobenzothiazole

Test item concentration: 50 (w/v) %

Test Animals			Control Animals		
ANIMAL NUMBER	SCORES OF DERMAL REACTION		ANIMAL NUMBER	SCORES OF DERMAL REACTION	
	24 hours after the patch removal	48 hours after the patch removal		24 hours after the patch removal	48 hours after the patch removal
86	0	0	89	0	0
87	0	0	91	0	0
90	0	0	92	0	0
93	0	0	96	0	0
94	0	0	97	0	0
98	0	0	101	0	0
99	0	0	111	0	0
100	0	0	112	0	0
102	1	1	113	0	0
103	0	0	119	0	0
104	1	1	-	-	-
105	0	0	-	-	-
106	1	1	-	-	-
107	1	1	-	-	-
108	0	0	-	-	-
109	0	0	-	-	-
114	0	0	-	-	-
115	0	0	-	-	-
116	0	0	-	-	-
117	0	0	-	-	-
mean of scores	0.20	0.20	mean of scores	0.00	0.00
number of positive/ number of tested	4/20	4/20	number of positive/ number of tested	0/10	0/10

Scoring of Skin Sensitization

0 = no visible change
1 = discrete or patchy erythema
2 = moderate and confluent erythema
3 = intense erythema and swelling

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

A9050B
propiconazole / fenpropidin EC (125/275)
SMO0G120

Batch Identification	**SMO0G120**
Product Code	**A9050B**
Other Product Code(s)	CGA64250/CGA114900 EC (125/275)

Chemical Analysis
(Active Ingredient Content)

– **Identity of the Active Ingredient(s)***	**confirmed**
– **Content of propiconazole ***	**13.6 % w/w corresponding to 129 g/l**
– **Content of fenpropidin ***	**29.2 % w/w corresponding to 278 g/l**

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

Methodology used for Characterization / Recertification: GC,

Physical Analysis

– **Appearance**	**brown, liquid**
– **Density ***	**952 kg/m^3**

Stability:

– **Storage Temperature**	**< 30°C**
– **Recertification Date**	**End of September 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: 121786

Study number(s) of batch recertification:

Authorisation: October 12, 2010

Dr. A.M. Dos Santos Alves
Analytical Development & Product Chemistry

APPENDIX 2 GLP Certificate

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

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1051 Budapest, Zrínyi u. 3.
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Ref. no: OGYI/8242-11/2010
Admin.: Urbin Magdolna Zita
Date: 16 December, 2010

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

LAB Research Kft.

(Base facility: H-8201 Veszprém, Szabadságpuszta, Hungary)

is able to carry out

physico-chemical testing, toxicity studies, mutagenicity studies, environmental toxicity studies on aquatic or terrestrial organisms, studies on behaviour in water, soil and air;bio-accumulation , safety pharmacology testing, 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: **4-8 October, 2010.**

Zsuzsanna Szepezdi, Ph. D.
Director-General