Propiconazole/Fenpropidin

Propiconazole/Fenpropidin EC (A9050B) - Primary Skin Irritation Study in Rabbits

Final Report Amendment 1

DATA REQUIREMENT(S): OECD 404 (2002)
EPA OPPTS 870.2500 (1998)
EC No 440/2008, B.4 (2008)

AUTHOR(S): Viktória Zelenák, M.Sc.

STUDY COMPLETION DATE: 21 June 2011

PERFORMING LABORATORY: LAB Research Ltd.
H-8200 Veszprém, Szabadságpuszta,
Hungary

LABORATORY PROJECT ID: Report Number: 10/290-006N
Study Number: 10/290-006N
Task Number: TK0037024

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

This study has been performed in accordance with the study plan, 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: Viktória Zelenák Date: 21 June 2011

Viktória Zelenák, M.Sc.
Study Director

Performing Laboratory: LAB Research Ltd.
H-8200 Veszprém, Szabadságpuszta,
Hungary

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

(FINAL REPORT AMENDMENT NO. 1.)

Study Number: 10/290-006N

Study Title: Propiconazole/Fenpropidin EC (A9050B) – Primary Skin Irritation Study in Rabbits

Test Item: Propiconazole/Fenpropidin EC (A9050B)

This study has been inspected, and the report as well as its amendment 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 the report accurately reflect the raw data produced during this study. All the corrections of or additions to the final report included in this amendment are considered to be represented accurately.

All inspections, data reviews and the report or report amendment audits were reported in writing to the study director and to management. The dates of such inspections and of the report audits are given below:

Date of Inspection	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
27 December 2010	Study Plan	03 January 2011	03 January 2011
04 January 2011	Treatment	04 January 2011	04 January 2011
16 February 2011	Draft Report	16 February 2011	16 February 2011
30 May 2011	Final Report	30 May 2011	30 May 2011
20 June 2011	Final Report Amendment 1	21 June 2011	20 June 2011

Signature: Heiderné Grób Ra Date: 21 June 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. the study titled "Propiconazole/Fenpropidin EC (A9050B) – Primary Skin Irritation Study in Rabbits" was performed, in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 21st June 2011

David J. Esdaile, M.Sc.
Scientific Director

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
Viktória Zelenák, M.Sc.	Study Director
Judit Tavaszi, M.Sc.	Assistant Scientist
Szabolcs Gáty, M.Sc.	Head of LAB Research Quality Assurance
Eric Yau	Syngenta Study Manager

Study dates

Experimental Starting Date:	04 January 2011
Experimental Completion Date	18 January 2011
Acclimatization:	29 December 2010 – 03 January 2011
Treatment:	04 January 2011
Observation of local findings:	Throughout 2 weeks after treatment. (18 January 2011)
Termination:	18 January 2011

Deviations from the guidelines

On occasions during the study the humidity (30-70%) was recorded out of the target range. The actual humidity range was 24-56 %.

This deviation has no impact on the outcome of the study.

Performing laboratory test substance reference number

100101

Other

The study documents:
- study plan,
- all raw data,
- sample of the test item,

- study report and any amendments,
- correspondence

will be archived according to the Hungarian GLP and to the LAB Research Ltd.'s standard operating procedures in the archives of LAB Research Ltd. 8200 Veszprém, Szabadságpuszta, Hungary.

After the retention time of 15 years has elapsed all the archived materials listed above will be returned to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded.

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

The primary skin irritation potential of Propiconazole/Fenpropidin EC (A9050B) was investigated according to the OECD test guideline no. 404. The animals were treated by topical semi-occlusive application of 0.5 mL of Propiconazole/Fenpropidin EC (A9050B) to the intact shaved flank of 3 young adult New Zealand White rabbits. The duration of treatment was 4 hours. The scoring of skin reactions was performed 1, 24, 48, 72 hours, 1 and two weeks after removal of the dressing. The primary irritation index was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of data points.

1.2 Results

The primary irritation index was 0.67.

At observations 1, 24, 48 and 72 hours after patch removal, very slight erythema and oedema (score 1) were observed in one animal. Very slight erythema (score 1) persisted in the same animal at the 1 week observation. Additionally, dry and cracked skin was also noted at 48 and 72 hours after the treatment in this animal. At the 1 and 2 week observation, dry and cracked skin or dry peeling skin were noted in all animals.

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

The study was terminated after the 2 week observation as no clinical signs were observed and all skin irritation effects had reversed.

The body weights of all rabbits were considered to be within the normal range of variability.

1.3 Conclusion

According to the Draize classification criteria Propiconazole/Fenpropidin EC (A9050B) is considered to be a “mild-irritant” to rabbit skin (P.I.I. = 0.67).

2.0 INTRODUCTION

This report issued on the 20 June 2011 is a reissue of the final report issued on the 30 May 2011 to correct the typographical error with the title heading, “TABLES SECTION”.

2.1 Purpose

The purpose of this primary skin irritation study was to assess the possible irritation potential when a single dose of Propiconazole/Fenpropidin EC (A9050B) was placed on the skin of rabbits for approximately 4 hours.

This study provides a rational basis for risk assessment in man as skin contact is one of the possible routes of human exposure.

As specified in the test guidelines, the test item was administered undiluted at 0.5 mL/animal.

2.2 Guidelines

The study was conducted according to the following guidelines:

OECD Guidelines for Testing of Chemicals, Section 4, number 404 “Acute Dermal Irritation / Corrosion”, adopted April 24, 2002.

United States Environmental Protection Agency, Health Effects Division Test Guidelines, OPPTS 870.2500 Acute Dermal Irritation EPA 712-C-98-196, August 1998.

Commission Regulation (EC) No 440/2008 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.4 Acute Toxicity: Dermal irritation/corrosion (Official Journal No L 142, 30 May 2008 p. 0182-0190)

2.3 Test Facility

This study was performed in an AAALAC-accredited laboratory. The Institutional Animal Care and Use Committee (IACUC) of LAB Research Ltd. reviewed the study plan and authorised the conduct of the study.

3.0 MATERIALS AND METHODS

3.1 Test Substance

Identification:	Propiconazole/Fenpropidin EC (A9050B)
Batch Number:	SMO0G120
Product Code:	A9050B
Purity:	Propicanozole – 129 g/L corresponding to 13.6 % w/w Fenpropidin – 278 g/L corresponding to 29.2 % w/w
Appearance:	Brown liquid
Density:	952 kg/m^3
Expiry Date:	End of September 2015
Storage Conditions:	< 30˚C
Safety Precautions:	Standard safety precautions (lab coat, gloves, goggles, face mask) were used in order to protect personnel health and safety.

The Certificate of Analysis is shown in Appendix 1.

3.2 Experimental Design

A volume of 0.5 mL (per animal) of Propiconazole/Fenpropidin EC (A9050B) was applied undiluted as supplied by the Sponsor.

According to Commission Directive 2004/73/EC, B.4. and OECD Guidelines 404, a test item does not need to be tested if the pH-value is less than 2 or greater than 11.5, owing to its predictable corrosive properties. The pH of the test item was measured before the study initiation date and was found to be 5.0.

Approximately 24 hours prior to the test the hair was clipped from the back and flanks of the animals with an electric clipper, exposing an area of approximately 100 cm^2 (10 cm x 10cm).

Animals with overt signs of skin injury or marked irritation which may have interfered with the interpretation of the results were not used in the test.

On the day of treatment, 0.5mL of Propiconazole/Fenpropidin EC (A9050B) was placed on a surgical gauze pad (*ca*. 2.5 cm x 2.5 cm). This gauze pad was applied to the intact skin of the clipped area and was kept in contact with the skin by a patch with a surrounding adhesive hypoallergenic plaster. The entire trunk of the animals was than wrapped with plastic wrap held in place with an elastic stocking.

The duration of treatment was 4 hours. The dressing was removed and the skin was flushed with lukewarm tap water to clean the application site.

Initially, a single animal was treated. As neither a corrosive effect nor a severe irritant effect was observed after the 1-hour exposure, the test was completed using the 2 remaining animals with an exposure period of 4 hours.

The viability/mortality was recorded daily from the day of application of the animals to the termination of test.

The clinical signs were recorded daily.

The body weights were recorded on the day of application and at termination of observation.

3.2.1 Animals

Animal species and strain:	Young Adult New Zealand White Rabbit
Breeder/supplier:	S&K-Lap Kft. 2173 Kartal, Császár út 135, Hungary
Number of animals per test:	3 (males)
Age at treatment:	~11 weeks
Identification:	The animals were identified by engraved ear tags. The cages were marked with individual identity cards with information about study number, sex, cage number, dose group and individual animal number.
Acclimatization:	Under laboratory conditions after health examination. Only animals without any visual signs of illness were used for the study.

3.2.2 Husbandry

Room number	618
Housing:	Animals were housed individually in metal cages.
Lighting periods:	12 hours daily, from 6.00 a.m. to 6.00 p.m.

Temperature:	17.7-21.9°C
Relative humidity:	24 -56%
Enrichment:	Rabbits were individually housed in AAALAC approved metal wire rabbit cages (65x65 cm with height of 45 cm). Cages are of an open wire structure and cages are placed together to allow some social interaction with rabbit(s) in adjoining cages.
Ventilation:	15-20 air exchanges/hour. The environmental parameters were recorded twice daily during the study and the acclimation period.
Diet	Animals received Purina Base – Lap gr. diet (Lot number: 0490 119) for rabbits produced by AgribrandsEurope Hungary PLC, H-5300 Karcag, Madarasi út, Hungary, *ad libitum*. Details of the diet are archived with the raw data.
Water	The animals received municipal tap water, as for human consumption, *ad libitum*, from an automatic system. 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 month, 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.

3.3 *Post Mortem* Investigations

At the end of the observation period, animals were euthanised by intramuscular injections of CP-Ketamin 10% and CP-Xylazine 2 % followed by i.v. Euthasol® 40% anaesthesia. Following confirmation of death, the carcasses were discarded without further examination. Details of materials employed for euthanasia are retained in the raw data and detailed in Section 3.3.1.

3.3.1 Materials used for euthanasia

Name: CP-Ketamin 10%
Batch No.: 10B18 3
Expiry Date: January 2012
Produced by: CP-Pharma, 31303 Burgdorf

Name: CP-Xylazine 2%
Batch No.: 10G06 2
Expiry Date: June 2013
Produced by: CP-PHARMA, 31303 Burgdorf

Name: Euthasol® 40%
Lot No.: 10C25 7
Expiry Date: February 2013
Produced by: AST Beheer B.V. Oudewater Netherlands
(Produlab Pharma, Raamsdonksveer)

3.4 Data Evaluation

The skin reaction was assessed according to the numerical scoring system listed in the Commission Directive 2004/73/EC, April 29, 2004 which was based on the Draize scoring system. The skin reaction was assessed at approximately 1, 24, 48, 72 hours, 1 week and 2 weeks after the end of exposure (removal of the dressing, gauze patch and test item).

Data were summarized in tabular form, showing for each individual animal the irritation scores for erythema and oedema at all measurement intervals. Any lesions were described, including the degree and nature of irritation, corrosion or any other toxic effects observed, and their reversibility.

The mean score was calculated across 3 scoring times (24, 48 and 72 hours after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately. An animal was positive when the mean score was 2 or greater. The test was positive for irritation when at least 2 animals were positive for the same endpoint (erythema/eschar or oedema).

The Cumulative Scores for the Skin Irritation Scores were calculated and represent the sum of all numerical scores for each animal at each time point. The resulting Mean Cumulative Skin Irritation Score was calculated for all animals at each time point.

The Primary Irritation Index (P.I.I.) was calculated by totaling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of available figures.

The irritation was classified according to the following criteria:

P.I.I. = 0	Not Irritant
$0 < P.I.I. \leq 2$	Mild irritant
$2 < P.I.I. \leq 5$	Moderate Irritant
$5 < P.I.I.$	Severe Irritant

Viability/mortality, clinical signs and dermal findings were recorded on data sheets.
Body weights were recorded at the beginning and at the end of experiment.
No statistical analysis was performed.

4.0 RESULTS AND DISCUSSION

4.1 Discussion

The primary irritation index was calculated by totaling the mean cumulative scores at 24, 48 and 72 hours for all animals and then dividing by the number of data points. The primary irritation index was 0.67 (out of a maximum score of 8.0).No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals.

At observations 1, 24, 48 and 72 hours after patch removal, very slight erythema and oedema (score 1) were observed in one animal. Very slight erythema (score 1) persisted in the same animal at the 1 week observation. Additionally, dry and cracked skin was also noted at 48 and 72 hours after the treatment in this animal. At the 1 and 2 week observation, dry and cracked skin or dry peeling skin were noted in all animals.

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

The study was terminated after the 2 week observation as no clinical signs were observed and all skin irritation effects had reversed.

The body weights of all rabbits were considered to be within the normal range of variability.

5.0 CONCLUSION

According to the Draize classification criteria Propiconazole/Fenpropidin EC (A9050B) is considered to be a “mild- irritant” to rabbit skin (P.I.I. = 0.67).

6.0 REFERENCES

Literature references listed are available upon request.

External references:

Draize, J.H. (1959): Dermal Toxicity. In Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, pp. 46-49. Austin, Texas: Association of Food and Drug Officials of the United States.

Draize, J.H., Woodward, G. & Calvery, H.O. (1944): Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exper. Therap. 83: 377-390.

TABLES SECTION

GLOSSARY FOR TABLE 1

Grading of Skin Reactions

ERYTHEMA AND ESCHAR FORMATION

No erythema	0
Very slight erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness)or eschar formation (injuries in depth preventing erythema) reading	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 (edges raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond the area of exposure)	4

TABLE 1 Skin Irritation Scores - Individual Values

Animal Number	Sex	Evaluation Interval*	Erythema	Oedema	Cumulative	
					Score	Mean
00766	male	1 hour	1	1	2.00	0.67
00768	male		0	0	0.00	
500	male		0	0	0.00	
00766	male	24 hours	1	1	2.00	0.67
00768	male		0	0	0.00	
500	male		0	0	0.00	
00766	male	48 hours	1	1	2.00	0.67
00768	male		0	0	0.00	
500	male		0	0	0.00	
00766	male	72 hours	1	1	2.00	0.67
00768	male		0	0	0.00	
500	male		0	0	0.00	
00766	male	1 week	1	0	1.00	0.33
00768	male		0	0	0.00	
500	male		0	0	0.00	
00766	male	2 weeks	0	0	0.00	0.00
00768	male		0	0	0.00	
500	male		0	0	0.00	

* Examinations were performed at the specified times after removal of the dressing.

TABLE 2 Skin Irritation Scores - Mean Values After 24, 48 and 72 Hours

Animal Number	Sex	Erythema	N	Oedema	N	Primary Skin Irritation Index
00766	male	1.00	3	1.00	3	0.67
00768	male	0.00	3	0.00	3	
500	male	0.00	3	0.00	3	
Mean score		0.33		0.33		
N= number of avaible data points.						

TABLE 3 Body Weights

Body weight (g)			
Animal No.	**Sex**	**Day of Treatment**	**Last Day of Observation**
00766	male	2797	3077
00768	male	2880	3346
500	male	2822	2974

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

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APPENDIX 2 Individual Local Findings

Animal No. 00766, Male

After 1 hour:	Erythema:	Very slight erythema (score1)
	Oedema:	Very slight oedema (score1)
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 24 hours:	Erythema:	Very slight erythema (score1)
	Oedema:	Very slight oedema (score1)
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 48 hours:	Erythema:	Very slight erythema (score1)
	Oedema:	Very slight oedema (score1)
	Flaking:	Dry cracked surface
	Staining:	No staining presented
After 72 hours:	Erythema:	Very slight erythema (score1)
	Oedema:	Very slight oedema (score1)
	Flaking:	Dry cracked surface
	Staining:	No staining presented
After 1 week:	Erythema:	Very slight erythema (score1)
	Oedema:	No abnormal findings noted
	Flaking:	Dry peeling surface
	Staining:	No staining presented
After 2 weeks:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	Dry peeling surface
	Staining:	No staining presented

APPENDIX 2 Individual Local Findings (Continued)

Animal No. 00768, Male

After 1 hour:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 24 hours:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 48 hours:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 72 hours:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 1 week:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	Dry peeling surface
	Staining:	No staining presented
After 2 weeks:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	Peeling surface
	Staining:	No staining presented

APPENDIX 2 Individual Local Findings (Continued)

Animal No. 500, Male

After 1 hour:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 24 hours:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 48 hours:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 72 hours:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	No abnormal findings noted
	Staining:	No staining presented
After 1 week:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	Dry cracked surface
	Staining:	No staining presented
After 2 weeks:	Erythema:	No abnormal findings noted
	Oedema:	No abnormal findings noted
	Flaking:	Peeling surface
	Staining:	No staining presented

APPENDIX 3 GLP Certificate

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

FŐIGAZGATÓ
1051 Budapest, Zrínyi u. 3.
tel: (1) 8869-320
fax: (1) 8869-480
e-mail: szepezdi.zsuzsanna@ogyi.hu

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

Translation (from Hungarian to English):
Stamp Translation = Országos Gyógyszerészeti Intézet (OGYI) = National Institute of Pharmacy
Főigazgató = Director-General