Bayer CropScience

2mEPSPS PROTEIN

IN VITRO DIGESTIBILITY STUDY IN

SIMULATED GASTRIC FLUID

DATA REQUIREMENT
No applicable guidelines

REPORT OF STUDY SA 06101

AUTHOR / STUDY DIRECTOR: D. ROUQUIE

TESTING FACILITY:

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STUDY COMPLETED ON: AUGUST 29, 2006

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA, Section 10(d)(1)(A)(B), or (C).

However, these data are the property of Bayer CropScience AG, and as such, are considered to be a trade secret and confidential for all purposes other than compliance with FIFRA 10.

Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any country other than the USA.

Company: Bayer CropScience
Research and Development Department

Company Agent:

Title:

Signature:

Date: _____

The above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study here reported was performed in accordance with the principles of Good Laboratory Practice ("Bonnes Pratiques de Laboratoire") described in the following issue, with the exception of the software Cary 100 used to measure the optical densities for assessing the activity of the pepsin. In addition, the dosing suspensions were not analyzed for concentration, homogeneity or stability.

- Organization for Economic Cooperation and Development (O.E.C.D.) Principles of Good Laboratory Practice, 1997 (January 26, 1998).

- European Directive 2004/10/EC (February 11, 2004).

- French decree n° 98-1312, on December 31, 1998 regarding Good Laboratory Practice.

- U.S. Environmental Protection Agency (E.P.A.)
  - 40 CFR part 160
    Federal Insecticide, Fungicide and Rodenticide Act (FIFRA);
    Good Laboratory Practice Standards: Final Rule, August 17, 1989.

- Good Laboratory Practice Standards for Toxicology studies on Agricultural Chemicals, Ministry of Agriculture, Forestry and Fisheries (M.A.F.F.) in Japan, notification 11 Nousan N°6283, October 01, 1999, modified by: notification 12 Nousan n°8628, December 06, 2000.

Author / Study Director:

Date: 29 August, 2006

D. ROUQUIE

Sponsor Representative:

Date: 29 August 2006

C. HERQUET-GUICHENEY

Study Submitter:

Date:

FLAGGING STATEMENTS

This page is reserved for flagging statements as may be required by E.P.A.

QUALITY ASSURANCE STATEMENT

The conduct of the study has been subjected to periodic inspections by the Bayer CropScience Sophia Antipolis Quality Assurance Unit. The types and dates of inspections and dates of reporting to study director and management are given below:

Type of Q.A. inspection	Date of Q.A. inspection	Date of reporting to Study Director	Date of reporting to Management
Protocol	April 28, 2006	May 02, 2006	May 11, 2006
SGF test	May 03, 2006	May 03, 2006	May 05, 2006
Report	July 28, 2006	July 31, 2006	August 29, 2006

This report has been audited by Quality Assurance personnel in accordance with the appropriate standardized operating methods. The reported results accurately reflect the original data of the study.

Quality Assurance Group Leader:

Date: August 29, 2006

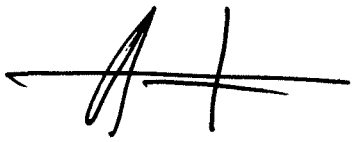
G. ODAGLIA

SIGNATURE

I, the undersigned, hereby declare that the work was performed under my supervision according to the procedures described and that this report provides a correct and faithful record of the results obtained.

There were no circumstances which affected the quality and integrity of the data.

Author / Study Director:



D. ROUQUIE

Date: 29 August 2006

STUDY PROFESSIONALS

The following professionals were involved in the conduct of this study:

AUTHOR / STUDY DIRECTOR	:	D. ROUQUIE
RESPONSIBLE TECHNICIAN	:	B. LABORY-CARCENAC
REPORT UNIT ASSISTANT	:	P. ALMERAS

TABLE OF CONTENTS

SUMMARY

The 2mEPSPS protein was tested for stability in human simulated gastric fluid (SGF) with pepsin at pH 1.2 for incubation times from 0.5 to 60 minutes. The protocol was in accordance with Thomas *et al*, (2004) protocol. Two reference proteins, horseradish peroxidase (HRP) and ovalbumin (OVA), were tested concurrently.

The reference proteins, HRP and OVA, were rapidly and slowly digested respectively, confirming the validity of this study.

The 2mEPSPS protein was degraded very rapidly in human simulated gastric fluid, within 30 seconds of incubation, in presence of pepsin, at pH 1.2.

INTRODUCTION

This *in vitro* human Simulated Gastric Fluid (SGF) digestibility study has been conducted to provide a full GLP study carried out in line with a current internationally recognized protocol. This study follows the protocol of the methods used in the recent ILSI ring trial (1). The method is based on the United States Pharmacopea (2).

In this study the test material was the 2mEPSPS protein. Two control proteins were included in this study horseradish peroxidase (HRP) and ovalbumin (OVA). They were chosen for this protocol as they are known to be rapidly and slowly digested, respectively. Results from several laboratories validate the choice of these two reference proteins (1).

The study protocol and amendments are presented in Attachment 1.

The study schedule was as follows:

Sponsor representative protocol approval date	April 27, 2006
Study initiation date*	April 27, 2006
Experimental starting date	May 03, 2006
Experimental completion date	July 19, 2006

* Date of protocol approval by Study Director

MATERIAL AND METHODS

1 - REAGENTS

1.1 Proteins

The test protein was supplied by the sponsor.

Identification	:	2mEPSPS protein
Batch N°	:	LEJ5837
Source	:	Bayer Bioscience N.V., Gent, Belgium
Description	:	Solution in storage buffer (100 mM Tris/HCl; 2.7 mM KCl; 137 mM NaCl; 1 mM DTT; pH 7.5), concentration 0.93 mg/ml
Purity	:	>95%
Expiry date	:	April 03, 2008
Storage	:	Approximately -20°C

The control proteins were purchased from an external supplier.

Identification	:	Horseradish peroxidase
Batch N°	:	111K74351
Source	:	Sigma, Reference P6782
Description	:	Red-brown powder
Stability	:	Stable under storage conditions until January 2007
Storage	:	Approximately -20°C

Identification	:	Ovalbumin, Albumin from chicken egg white (OVA)
Batch N°	:	031K7025
Source	:	Sigma, Reference A5503
Description	:	White powder
Stability	:	Stable under storage conditions until June 2006
Storage	:	Approximately 4°C

The certificates of analysis are presented in Attachment 2.

1.2 Proteolytic enzyme

Identification	:	Pepsin (from porcine gastric mucosa)
Batch N°	:	074K77164
Source	:	Sigma, Reference P6887
Description	:	Off-white powder
Activity	:	3300 Units /mg protein
Stability	:	Stable under storage conditions until November 2006
Storage	:	Approximately -20°C

The certificate of analysis is presented in Attachment 2.

2 - TEST SOLUTIONS

2.1 Protein solutions

The test protein was tested at a concentration of 0.93 mg/ml in storage buffer solution (100 mM Tris/HCl; 2.7 mM KCl; 137 mM NaCl; 1 mM DTT; pH 7.5). The two reference proteins were prepared at 0.93 mg/ml in storage buffer solution.

2.2 Digestive solution

The human simulated gastric fluid (SGF) consisted of the following:

- 100 ml distilled water
- 400 mg NaCl
- 1.68 ml 10N HCl
- Titrated to pH 1.2 using dilute NaOH then the volume adjusted to 200 ml with distilled water.

The pepsin was added at the above solution to provide a final concentration of 10 units of pepsin per µg test protein.

In order to validate the activity of the pepsin solution, a quality control procedure was carried out to demonstrate the rate of denaturation of hemoglobin. The pepsin solution was found to have a normal level of activity based on historical data obtained during the ILSI ring trial test (1).

3 - DIGESTION INCUBATION

The protein incubation for the test and control materials was made in 2 ml microcentrifuge tubes in a waterbath at approximately 37°C. For each test and control protein solution, 80 µl was added to 1520 µl of SGF and mixed. Samples of 200 µl were taken at 0.5, 2, 5, 10, 20, 30 and 60 minutes. The tubes were agitated after each sampling and at 45 minutes.

As soon as samples were taken, the reaction was terminated by adding the 200 µl sample to a tube containing 70 µl 200 mM $NaHCO_3$ (pH 11.0).

Additional control samples were prepared:

- a zero minute incubation of protein (10 µl) with 'SGF without any pepsin' (190 µl);
- a 60 minute incubation of protein (10 µl) with 'SGF without any pepsin' (190 µl);
- a 'time zero' sample was produced by adding the protein (10 µl) to SGF (190 µl) after the reaction was terminated as above;
- a sample of SGF alone before incubation and the reaction terminated as above;
- a sample of SGF alone after 60 minutes incubation and the reaction terminated as above.

Aliquots of 20 or 40 µl of the samples were used for analysis on SDS-PAGE gels and the remaining was frozen at -20°C.

4 - SDS-PAGE ELECTROPHORESIS

Gel electrophoresis was carried out, following the method of Laemmli (3) using a BioRad Mini-Protean III cell. Prior to running SDS-PAGE, 5 µl or 10 µl of 5X Leammli solution was added to 20 µl or 40 µl of digestion samples and heated for 10 minutes at more than 90°C before loading the gel. Samples of 15 µl were added to wells of an SDS-PAGE gel (10 well, 1mm 10-20% gradient polyacrylamide Tris/Tricine)(BioRad, France).

A suitable marker solution (2.5-200 kDa) was used to provide reference points of known molecular weights on the gel (Mark 12, Invitrogen, France).

A setting of 117 ± 7 volts was used at a constant voltage for approximately 2 hours to give gels with a correct appearance (near straight lines for sample front and a front finishing near the top of the gel).

Gels were stained by a sensitive Coomassie blue method (Invitrogen, France) based on the work of Neuhoff *et al.* (4). After rinsing, the gels were scanned using a GS800 scanner (Biorad, France).

5 - DATA STORAGE

All raw data, supporting documents, as well as protocol, protocol amendments and final report are maintained in the document archive room. All of the above will be archived for at least 10 years in the designated areas at:

Bayer CropScience
355, rue Dostoïevski
BP 153
06903 Sophia Antipolis Cedex
France

RESULTS AND DISCUSSION

1 - 2mEPSPS PROTEIN (Fig. 1)

The SDS-PAGE analysis of the 2mEPSPS protein solution showed one band located below the molecular weight marker at 55.4 kDa. The 2mEPSPS protein band was visible in the zero and 60 minute incubation times without pepsin, with no decrease in stain intensity at 60 minutes. At time zero of incubation with SGF, the 2mEPSPS protein and the pepsin bands at approximately 36 kDa were clearly visible. At 30 seconds and all subsequent incubation times, there were no 2mEPSPS band and no smaller bands. This indicates a complete digestion of the 2mEPSPS proteins (encoded by the *2mepsps* gene) within 30 seconds.

The pepsin band at approximately 36 kDa showed no decrease in stain intensity at 60 minutes, indicating that the pepsin was stable.

2 - HORSERADISH PEROXIDASE UNSTABLE REFERENCE PROTEIN (Fig. 2)

The horseradish peroxidase (HRP) protein was visible at approximately 46 kDa in the zero and 60 minute incubation times without pepsin, with no decrease in stain intensity at 60 minutes. At time zero of incubation with SGF, the HRP protein and the pepsin at approximately 36 kDa bands were clearly visible. At 30 seconds and subsequent incubation times, there was no HRP band at 46 kDa, and no smaller bands. This indicates a complete digestion of the horseradish peroxidase unstable reference protein within 30 seconds.

The pepsin at approximately 36 kDa band showed no decrease in stain intensity at 60 minutes, indicating that the pepsin was stable.

3 - OVALBUMIN STABLE REFERENCE PROTEIN (Fig. 3)

The ovalbumin (OVA) protein was clearly visible at approximately 45 kDa in the zero and 60 minute incubation time without pepsin, with no decrease in stain intensity at 60 minutes. At time zero of incubation with SGF, the OVA (at approximately 45 kDa) and the pepsin (at approximately 36 kDa) bands were clearly visible. At 30 seconds, 2 and 5 minutes incubation times, the 45 kDa band had not diminished, but at subsequent times, a gradual reduction in the staining at 45 kDa was apparent. At 60 minutes, the 45 kDa band was still visible. This indicates that the OVA stable reference protein was not totally digested within 1 hour.

The pepsin at approximately 36 kDa band showed no decrease in stain intensity at 60 minutes, indicating that the pepsin was stable.

4 - DISCUSSION

It has been shown that the pepsin was active before the study was carried out, and that two reference proteins, horseradish peroxidase and ovalbumin, were rapidly and slowly digested respectively. The results of the reference proteins are in line with the results obtained in an international ring trial organized by ILSI (1). These quality control procedures confirm that the study procedures and reagents were adequate to detect the rate of digestion of proteins in this simulated gastric fluid (SGF) study.

The 2mEPSPS protein was degraded very rapidly with no residual protein visible at 30 seconds of incubation with SGF, in presence of pepsin, at pH 1.2. There was no significant digestion at pH 1.2 in the absence of pepsin, showing that digestion requires pepsin.

CONCLUSION

The 2mEPSPS protein was degraded very rapidly in human simulated gastric fluid, within 30 seconds of incubation, in presence of pepsin, at pH 1.2.

PROTOCOL DEVIATIONS

There were no protocol deviations during the study.

Author / Study Director:

Date: 29 August, 2006

D. ROUQUIE

REFERENCES

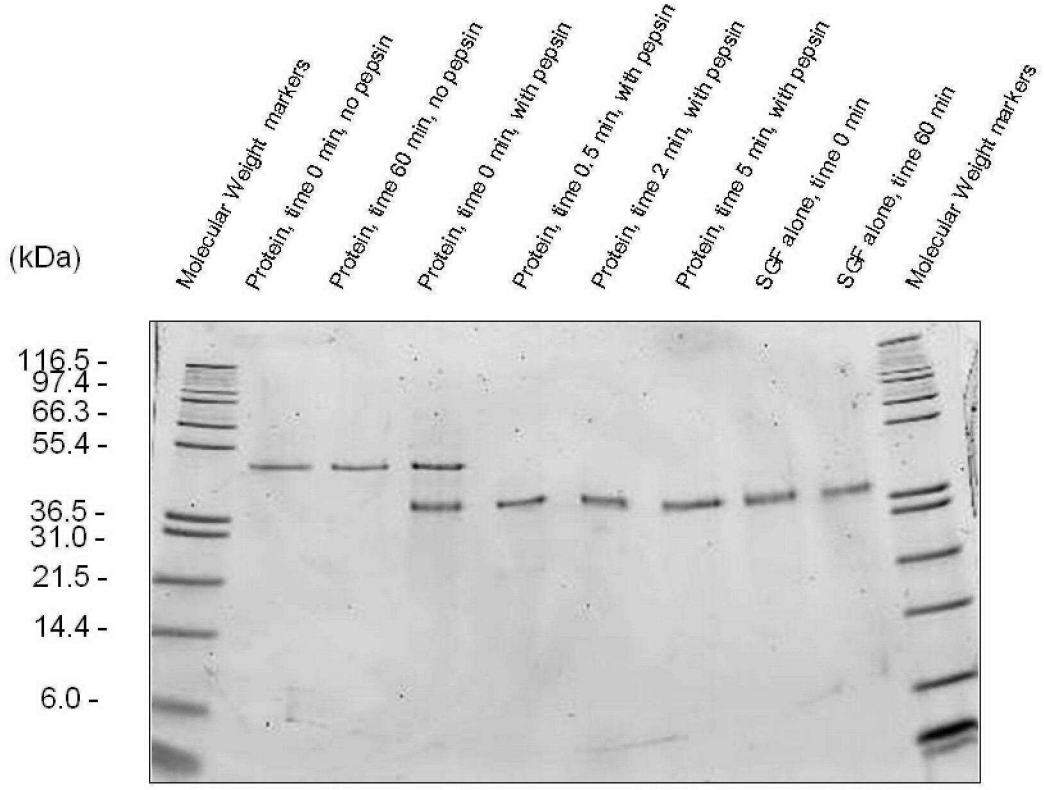
	DART Numbers	
1	M-229898-01-1	THOMAS et al. (2004). A multi-laboratory evaluation of a common *in vitro* pepsin digestion assay protocol used in assessing the safety of novel proteins. *Regulatory Toxicology and pharmacology*, **39**, pp. 87-98.
2	M-273056-01-1	United States Pharmacopea (1990) Vol XXII, pp. 1788-1789, United States Pharmacopeia Convention, Inc, Rockville, MD.
3	M-223866-01-1	LAEMLLI U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970, **227**, pp. 680-5.
4	M-273931-01-1	NEUHOFF V., AROLD N., TAUBE D., EHRHARDT W. (1988): Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis*, **9**, pp. 255-62.

ABBREVIATIONS

%	Percentage
°C	Degree (s) Celcius
µg	Microgram (s)
µl	Microliter (s)
EDTA	Ethylendiamineteraacetic acid
g	Gram (s)
g/ml	Gram/milliliter
GLP	Good Laboratory Practice
HRP	Horseradish Peroxidase
ILSI	International Life Science Institute
kDa	Kilodalton
kg	Kilogram (s)
M	Molar
mg	Milligram
mg/ml	Milligram/milliliter
Min	Minute (s)
ml	Milliliter
mM	Millimolar
OVA	Ovalbumin
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
pH	Potential of hydrogen
SDS	Sodium dodecyl sulfate
SGF	Human simulated gastric fluid
USA	United States of America
w/v	Weight/volume

FIGURES

Fig. 1


(kDa)
Molecular Weight markers
Protein, time 0 min, no pepsin
Protein, time 60 min, no pepsin
Protein, time 0 min, with pepsin
Protein, time 0.5 min, with pepsin
Protein, time 2 min, with pepsin
Protein, time 5 min, with pepsin
SGF alone, time 0 min
SGF alone, time 60 min
Molecular Weight markers
116.5 -
97.4 -
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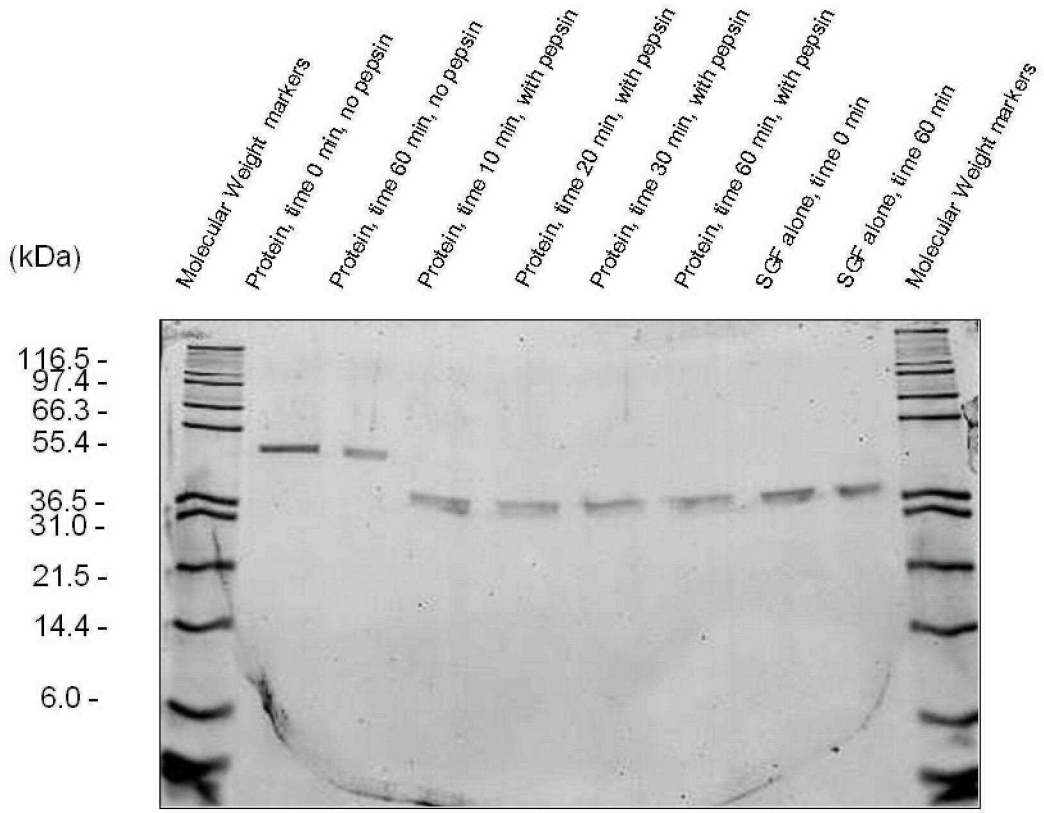
55.4 -
36.5 -
31.0 -
21.5 -
14.4 -
6.0 -


(kDa)
Molecular Weight markers
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Protein, time 60 min, no pepsin
Protein, time 10 min, with pepsin
Protein, time 20 min, with pepsin
Protein, time 30 min, with pepsin
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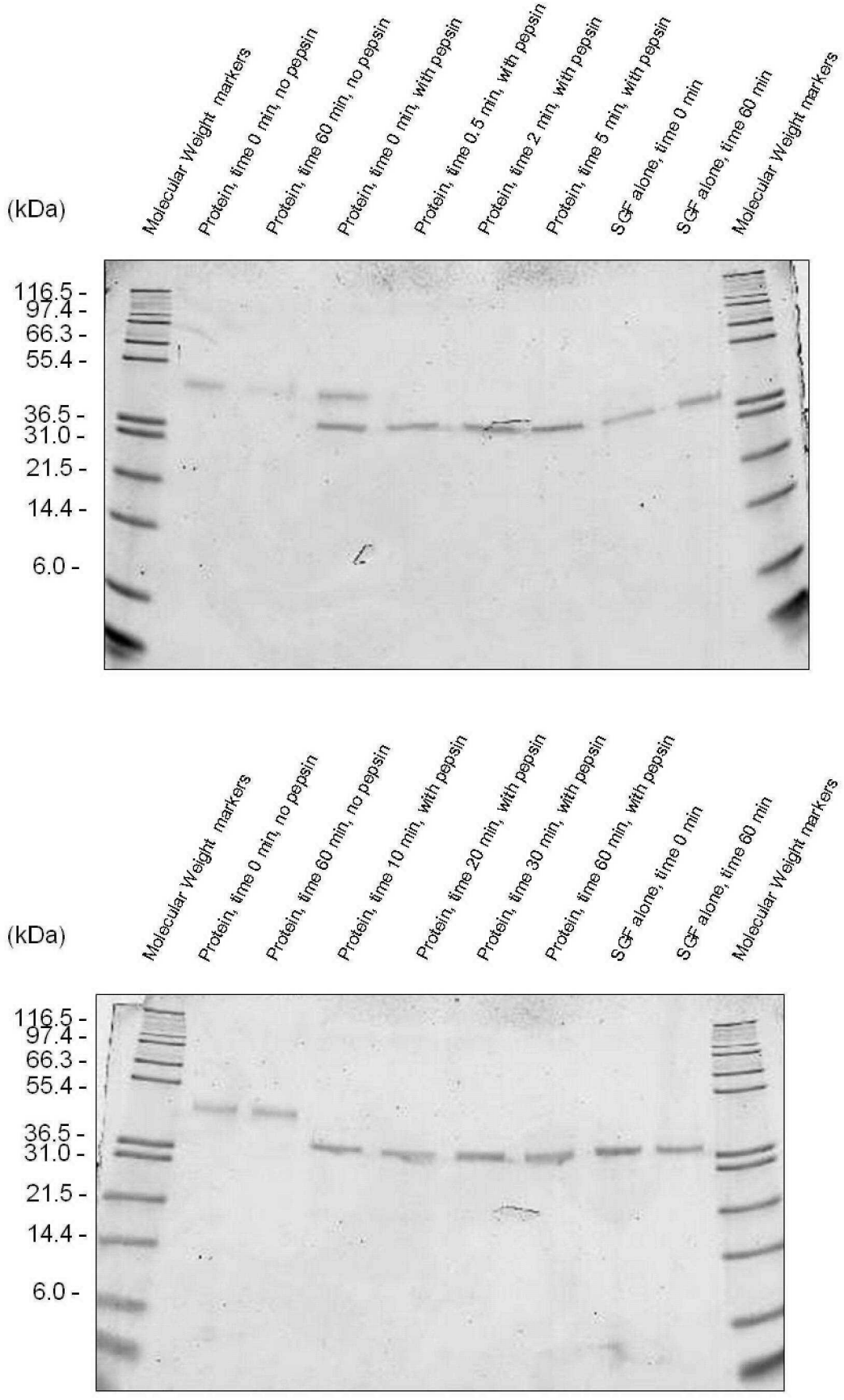
116.5 -
97.4 -
66.3 -
55.4 -
36.5 -
31.0 -
21.5 -
14.4 -
6.0 -

Fig. 2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Protein, time 10 min, with pepsin
Protein, time 20 min, with pepsin
Protein, time 30 min, with pepsin
Protein, time 60 min, with pepsin
SGF alone, time 0 min
SGF alone, time 60 min
Molecular Weight markers
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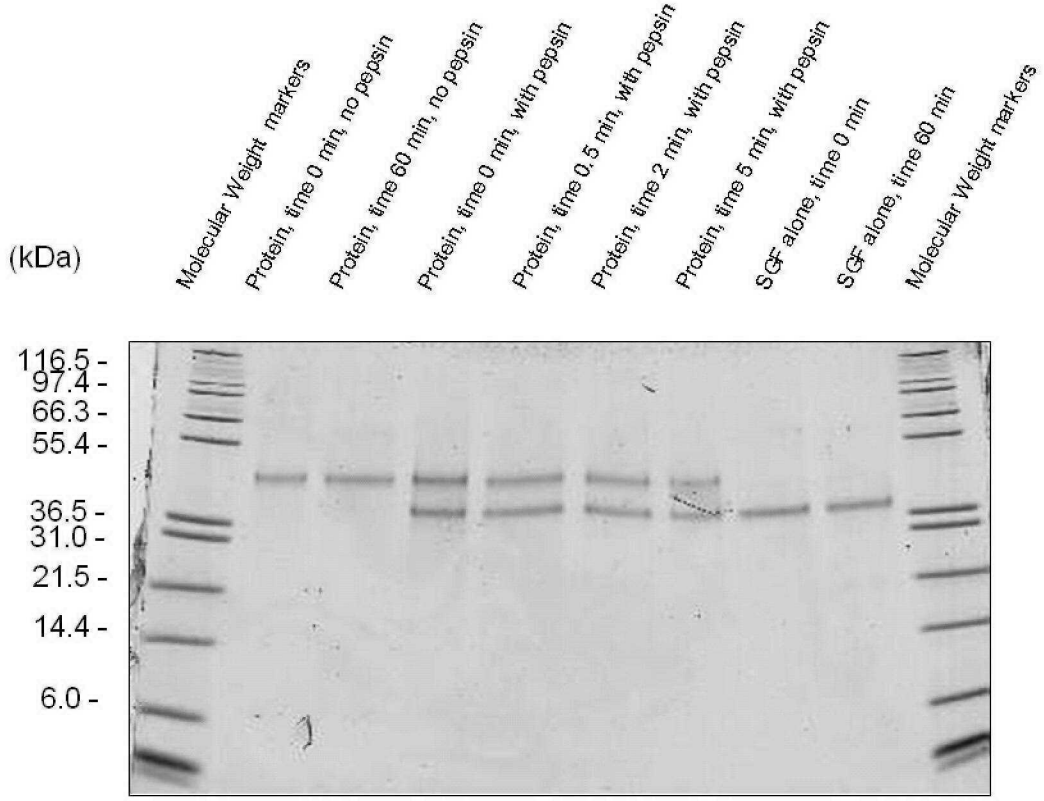
97.4 -
66.3 -
55.4 -
36.5 -
31.0 -
21.5 -
14.4 -
6.0 -

Fig. 3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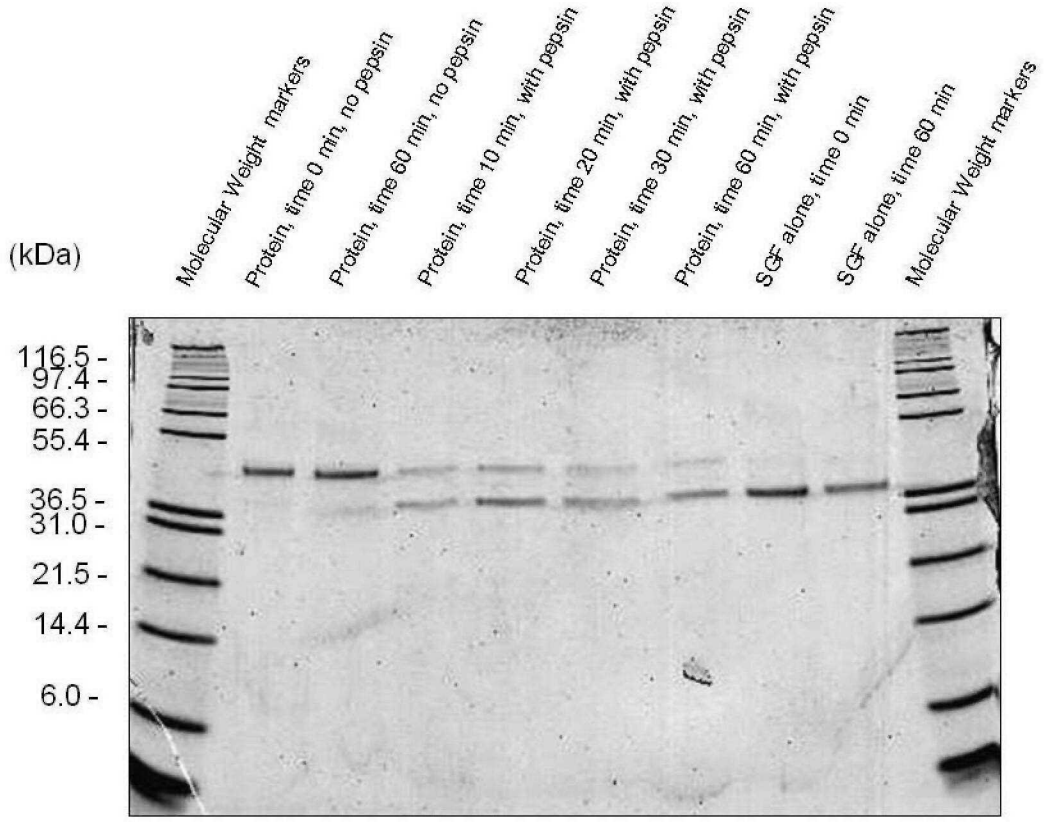
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Molecular Weight markers
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Protein, time 10 min, with pepsin
Protein, time 20 min, with pepsin
Protein, time 30 min, with pepsin
Protein, time 60 min, with pepsin
SGF alone, time 0 min
SGF alone, time 60 min
Molecular Weight markers
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97.4
66.3
55.4
36.5
31.0
21.5
14.4
6.0

ATTACHMENTS

ATTACHMENT 1 - **PROTOCOL AND AMENDMENT**

2mEPSPS protein

***IN VITRO* DIGESTIBILITY STUDY IN SIMULATED GASTRIC FLUID**

TESTING FACILITIES

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1 - GENERAL

1.1 PURPOSE OF STUDY

The present study is designated to determine the stability of the 2mEPSPS protein, encoded by *2mepsps* gene, in human simulated gastric fluid. The rate of *in vitro* protein degradation will be observed, which will give an indication of the gastric stability of the protein. The control proteins horseradish peroxidase and ovalbumin were chosen as they are known to be respectively rapidly and slowly digested,.

1.2 GOOD LABORATORY PRACTICE COMPLIANCE

This study will be performed in accordance with the principles of Good Laboratory Practice ("Bonnes Pratiques de Laboratoire") described in the following issues, with the exception of the control and test material expiry dates and that the dosing suspensions were not analyzed for concentration, homogeneity and stability:

-O.E.C.D. (Organization for Economic Cooperation and Development) Principles of Good Laboratory Practice, 1997 (January 26, 1990).

-European Directive 2004/10/EC (February 11, 2004).

-U.S. E.P.A. (Environmental Protection Agency)
. 40 CFR Part 160
Federal Insecticide, Fungicide and Rodenticide Act (FIFRA);
Good Laboratory Practice Standards: Final Rule, August 17, 1989.

-Good Laboratory Practice Standards for Toxicology studies on Agricultural chemicals, Ministry of Agriculture, Forestry and Fisheries (M.A.F.F.) in Japan, notification 11 Nousan N°6283, October 01, 1999, modified by 12 Nousan N°8628, December 06, 2000.

-French Decree N°98-1312 regarding Good Laboratory Practice, December 31, 1998.

1.3 REGULATORY GUIDELINES

No data requirement for this study.

1.4 QUALITY ASSURANCE

The Quality Assurance Unit of Bayer CropScience, 355 rue Dostoïevski, BP 153, 06903 Sophia Antipolis Cedex, France, will undertake and document inspections while the study is in progress and will audit the study report.

2 - STUDY PERSONNEL

2.1 STUDY DIRECTOR:

Date: April 27, 2006 D. ROUQUIE

2.2 SPONSOR REPRESENTATIVE:

Date: April 27, 2006 C. HEROUET-GUICHENEY

2.3 OTHER STUDY PERSONNEL

Responsibility	**Name**
In-life Supervisor	JAUSSELY Catherine
Responsible Technician	LABORY-CARCENAC Bénédicte

Other study personnel will be identified as appropriate in the study file.

3 - PROPOSED DATES

Experimental starting date : May 03, 2006
Experimental completion date : May 31, 2006 (estimated)

4 - OVERVIEW OF STUDY DESIGN

The method described in this protocol is based on Thomas *et al.* (2004) publication.
A solution of the test protein will be incubated with simulated gastric fluid (a pepsin solution at pH 1.2) at 37±1°C and samples taken for analysis at time-points of 0, 0.5, 2, 5, 10, 20, 30 and 60 minutes. The resultant protein solution will be analysed for presence of the parent protein and stable protein fragments by SDS-PAGE electrophoresis. The gel will be stained with Coomassie blue and scanned. Appropriate controls will allow an evaluation of the rate and degree of digestion under these test conditions. Controls will include protein in acid without pepsin, SGF without protein, and parallel testing of proteins known to be digested rapidly and slowly.

5- MATERIALS AND METHODS

5.1 PROTEINS AND PEPSIN

The pepsin used will be from Sigma, reference P-6887, batch 074K77164 with a nominal activity of 3300 units per mg pepsin.

The test protein will be supplied by the Sponsor (Bayer BioScience NV, Gent Belgium). The certificate of analysis will be attached to the final report;

Test Protein name: 2mEPSPS protein (encoded by the *2mepsps* gene)
Batch N°: LEJ5837
Purity >90 % 2mEPSPS

Storage: approximately – 20 ° C.

Certified through at least 6 months.

The 'rapid digestion' protein control is horseradish peroxidase (Sigma, batch N°111K74351).

The 'slow digestion' protein control is ovalbumin (Sigma, batch N°31K7025).

5.2 PROTEIN SOLUTIONS

The 2mEPSPS protein will be received as a stock solution of 0.931 mg/ml in storage buffer (0.1 M Tris/HCl; 2.7 mM KCl; 137 mM NaCl; 1 mM DTT; pH 7.5).

Each control protein will be prepared as a stock solution of 0.931 mg/ml in storage buffer (0.1M Tris/HCl; 2.7 mM KCl; 137 mM NaCl; 1 mM DTT; pH 7.5).

Control protein solutions may be prepared fresh or stored frozen at -20°C or colder (thaw only once and ensure protein is dissolved by visual inspection).

5.3 SIMULATED GASTRIC FLUID

The gastric control solution (G-con) will be prepared as follows:
400 mg NaCl
1.68 ml 10N HCl
Titrate to pH 1.2 using dilute HCl
Add distilled water to a volume of 200 ml.

This solution may be stored for up to 4 months at 4°C.

The above G-con will be mixed with the pepsin at a concentration to give 10 pepsin activity units per µg of test protein and then used within 2.5 hours (being kept on ice when not in use).

Example of dilution calculation: For a batch of pepsin with a nominal activity of 3 460 units/mg;

3.8 mg pepsin would be mixed with 5 ml G-con (13 148 units of activity)

i.e. 4 000 units per 1 520 µl will be added to 400 µg protein (80 µl of a 5 mg/ml protein solution). This is 10 units per µg of protein.

To ensure that the pepsin is active, it will be tested against a pepsin substrate, hemoglobin (Sigma, batch N°50K7415). This test must show that the pepsin is able to degrade hemoglobin rapidly. The method will be documented.

5.4 5X LAEMMLI BUFFER

5X Laemmli buffer contains: 40% glycerol, 5% β-mercaptoethanol (BME), 10% SDS, 0.33 M Tris-HCl; 0.05% bromophenol blue (adjusted to pH 6.8). The mix without the BME is stable for one year, after addition of BME solution is stable for 1 month when stored at room temperature.

5.5 DIGESTION

A 2 ml microcentrifuge tube of SGF for the serial digestion samples, and a series of control tubes will be warmed to approximately 37°C in a waterbath for approximately 2 minutes before the time zero (addition of protein and/or sampling).

The 'serial digestion' tube will be for the protein digestion and will contain 1 520 µl of the SGF for sampling from 0.5 to 60 minutes.

Eighty µl of protein solution will be added (time zero) and briefly vortexed then returned to the waterbath.

At 0.5, 2, 5, 10, 20, 30 and 60 minutes, samples of 200 µl will be taken from tube. Prior to each sampling, tubes will be briefly vortexed. An additional vortexing at 45 minutes without sampling will be performed.

Control tubes:

G-con + protein time 0	190µl G-con (SGF without pepsin) + 10 µl protein : sample at time zero
G-con + protein time 60 min	190 µl G-con (SGF without pepsin) + 10 µl protein : sample at 60 minutes (vortex and incubate in waterbath)
SGF + protein time 0	190 µl SGF + 70 µl of 200 mM $NaCO_3$ (pH 11.0) + 70 µl 5X Laemmli buffer + 10 µl protein (added last after mixing) : sample at time zero
SGF alone, 0 min	190 µl SGF + 10 µl distilled water: sample at time zero
SGF alone, 60 min	190 µl SGF + 10 µl distilled water: sample at 60 minutes (vortex and incubate in waterbath)

5.6 SAMPLES

The reaction will be stopped as soon as samples are taken.

For the serial samples, the reaction will be terminated by adding the 200 µl of digestion sample to a tube containing 70 µl 200 mM $NaHCO_3$ (pH 11.0).

Samples may be directly used for analysis on SDS-PAGE gels or frozen at -20°C.

5.7 SDS-PAGE ANALYSIS

The method will be based on that of Laemmli (1970) using a BioRad Mini-Protean III cell. Prior to running SDS-PAGE, 5 µl of 5X Leammli solution will be added to 20 µl of digestion samples and heated for 10 minutes at >90°C before loading the gel. Samples of 15 µl will be added to wells of an SDS-PAGE gel (15 wells, 1mm 10-20% gradient polyacrylamide Tris-Tricine) (BioRad, France). A few grains of sucrose may be added to the samples at the last heating stage if a higher density is needed to allow correct deposition of samples into the wells.

A suitable marker solution (2.5-200 kDa) will be used to provide reference points of known molecular weights on the gel (Mark 12, Invitrogen, France).

Loading order on gel:

Lane	Sample
1	Markers
2	G-con + protein time 0
3	G-con + protein time 60 min
4	SGF + protein time 0
5	SGF + protein time 0.5 min
6	SGF + protein time 2 min
7	SGF + protein time 5 min
8	SGF + protein time 10 min
9	SGF + protein time 20 min
10	SGF + protein time 30 min
11	SGF + protein time 60 min
12	SGF alone, 0 min
13	SGF alone, 60 min
14	Markers
15	empty

A setting of 117 ± 7 volts at a constant voltage for approximately 90 minutes will be selected to give gels with a correct appearance (near straight lines for sample front and a front finishing near the top of the gel).

Gels will be stained by the Coomassie blue method and the gels will be scanned. If necessary, a silver staining or a western blot analysis will be performed. The gels are not stable for more than a week, so the raw data will be the scanned image.

5.8 DATA ANALYSIS

The results of a detailed visual inspection of the scans will be reported.

The gel will be considered to be valid if:

- the pepsin blot is at the correct molecular weight (approximately 36 kDa) and is stable throughout the study.
- the protein blot is clearly visible at time zero (lanes 2 and 4) and is not visible in the lanes without protein (lanes 12 and 13).
- at least one marker lane is clearly visible to allow a molecular weight evaluation.

The study will be considered valid if the gels are valid and the control proteins give results that are in line with expected data.

If stable fragments of the protein are visible on the gel, then their intensity and time-course will be reported.

The time at which the parent protein disappears will be reported as the time for protein digestion.

6 - STATISTICAL ANALYSES

No statistical analysis will be performed.

7 - REPORTING

7.1 INTERIM REPORTS

Any unexpected findings during the course of the study will be reported to the Sponsor Representative.

7.2 FINAL REPORT

A copy of the draft report will be submitted to the Sponsor Representative and the Quality Assurance Unit for review. With the exception of the dated signature of scientists and other professional personnel, the draft report will contain all information and data to be included in the final report. The final report will include the information and data required by current internationally recognized regulations.

8 - ARCHIVING

All raw data, supporting documents as well as protocol, protocol amendments, an aliquote of the formulated test substance and final report will be maintained in the archive room.

All of the above will be archived for at least 10 years in the designated areas at:

Bayer CropScience
355, rue Dostoïevski
BP 153
06903 Sophia Antipolis Cedex
France

9 - REFERENCES

THOMAS *et al.*, (2004). A multi-laboratory evaluation of a common *in vitro* pepsin digestion assay protocol used in assessing the safety of novel proteins. *Regulatory Toxicology and Pharmacology*, **39**, pp. 87-98.

LAEMMLI, U.K., (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 1970, **227**, pp. 680-5.

PROTOCOL AMENDMENT

Protocol SA 06101

2mEPSPS PROTEIN
***IN VITRO* DIGESTIBILITY STUDY IN SIMULATED GASTRIC FLUID**

Protocol amendment: N°1

Reason(s):
Reason 1- Typing Error
In paragraph 1.2, the date of the O.E.C.D. (Organization for Economic Cooperation and Development) Principles of Good Laboratory Practice, 1997 should read as (January 26, 1998) and not (January 26, 1990) as stated in the Study Protocol.

Reason 2 – Modification of the SDS-PAGE analysis procedure
As the BioRad 15-well SDS-PAGE gels are not available anymore from the supplier, they will be replaced by Biorad SDS-PAGE gels (10 wells, 1mm 10-20% gradient polyacrylamide Tris-Tricine). As a consequence, all the samples will be loaded on two gels and the sample loading order on the gels will be as follows:

Gel 1

Lane	Sample
1	Markers
2	G-con + protein time 0
3	G-con + protein time 60 min
4	SGF + protein time 0
5	SGF + protein time 0.5 min
6	SGF + protein time 2 min
7	SGF + protein time 5 min
8	SGF alone, 0 min
9	SGF alone, 60 min
10	Markers

Gel 2

Lane	Sample
1	Markers
2	G-con + protein time 0
3	G-con + protein time 60 min
4	SGF + protein time 10 min
5	SGF + protein time 20 min
6	SGF + protein time 30 min
7	SGF + protein time 60 min
8	SGF alone, 0 min
9	SGF alone, 60 min
10	Markers

Study Director:

Date: May 09, 2006

D. ROUQUIE

ATTACHMENT 2 - **CERTIFICATES OF ANALYSIS**

Title

Certificate of analysis for the 2mEPSPS protein produced in *E. coli*

Batch n°: LEJ5837

Author

Nadine Bautsoens
Koen Hendrickx, Ph.D.

Completed On
June 16, 2006

Testing Facility

Molecular & Biochemical Analytical Services
Expression and Protein Characterization
Bayer BioScience N.V.
Technologiepark 38
B-9052 Gent
Belgium

Study number

BBS06-003

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

This report is confidential. No part of the report or any information contained herein may be disclosed to any third party without the written prior authorisation of Bayer BioScience N.V.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The undersigned hereby declare that the work to which this report refers was performed according to the procedures herein described and this report provides an accurate record of the results obtained. The study was conducted in accordance with the Good Laboratory Practice Standards as specified in the OECD/EU principles of Good Laboratory Practice except for the following:

1. No validation of computerized systems attached to: multifunctional monochromator Tecan Safire², and autoclave was performed.

Study Director

Koen Hendrickx
Expression and Protein Characterization
Molecular and Biochemical Analytical Services

12/06/06
Date

STUDY IDENTIFICATION PAGE

Study start date:	April 3th, 2006
Experimental start date:	April 10th, 2006
Experimental Termination date:	May 5th, 2006
Study Completion date:	June 16, 2006
Test Facility Address:	Bayer BioScience N.V. Molecular & Biochemical Analytical Services GLP Test Facility Technologiepark 38 9052 Gent – Belgium Tel: +32 9-243 04 11 Fax: +32 9-224 06 94
Test Facility Manager:	Elizabeth Bates Address see Test Facility Tel: +32 9-243 04 25 Fax: +32 9-224 06 94 e-mail: elizabeth.bates@bayercropscience.com
Study Director:	Koen Hendrickx Address see Test Facility Tel: +32 9-243 04 40 Fax: +32 9-224 06 94 e-mail: koen.hendrickx@bayercropscience.com
Study Personnel:	Nadine Bautsoens Address see Test Facility Tel: +32 9-243 05 86 Fax: +32 9-224 06 94 e-mail: nadine.bautsoens@bayercropscience.com
Sponsor Representative:	Dominique Rouan Global Regulatory Affairs Manager Regulatory Affairs Address see Test Facility Tel: +32 9-243 04 21 Fax: +32 9-233 19 83 e-mail: dominique.rouan@bayercropscience.com
Study Director in Training:	Nadine Bautsoens Address see Test Facility Tel: +32 9-243 05 86 Fax: +32 9-224 06 94 e-mail: nadine.bautsoens@bayercropscience.com

QUALITY ASSURANCE STATEMENT

Report **BBS06-003** Date: 12 JUN 2006
Page **5**

Quality Assurance (GLP)

Quality Assurance Statement

Title: **Certificate of analysis for the 2mEPSPS protein produced in *E. coli*.**

Study: BBS06-003

This study was periodically inspected and properly signed records of these inspections were submitted to testing facility management and the study director as shown below.
This report has been audited by the GLP Quality Assurance. The reported results accurately reflect the original data of the study.

Phase of Study	Inspection	Report
Study plan	06 APR 2006	06 APR 2006
Study plan amendment	07 APR 2006	07 APR 2006
Study conduct	10 APR 2006	10 APR 2006
	24 APR 2006	25 APR 2006
	04 MAY 2006	05 MAY 2006
Draft report	01 JUN 2006	02 JUN 2006
Final report	12 JUN 2006	12 JUN 2006

S. Berghman
GLP Quality Assurance

12/06/06

APPROVALS PAGE

Study Director / Author	Koen Hendrickx	Date 16/06/06
Test facility management	Elizabeth Bates	Date 13/06/06
Sponsor representative	Dominique Rouan	Date 13 June 2006
Head of MBAS	Henk Joos	Date 16 June 2006 HJ

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SUMMARY

This study was undertaken to confirm the identity of the bacterially produced 2mEPSPS to be used in subsequent studies and to determine the concentration and purity of the produced protein.

Five tests were performed in this study: (1) The concentration of the protein was determined by the Bradford technique. (2) The molecular weight of the protein was estimated by SDS-PAGE. (3) The same SDS gel was used to estimate the purity of the test item. (4) The immunological relationship with 2mEPSPS was tested by western blotting. (5) The activity of the protein was determined in an activity assay.

The identity of the 2mEPSPS protein was confirmed based on the observed molecular weight, cross-reactivity with the antibodies previously raised towards 2mEPSPS and the activity of the protein. The concentration of the protein was estimated to be 0.93 mg/ml and the purity was estimated to be 99.52%.

1.OBJECTIVE

In this study, the purified 2mEPSPS protein produced in *Escherichia coli* was analyzed for its concentration, identity and purity.

2. OVERVIEW OF EXPERIMENTAL DESIGN

Five analyses were performed on test item T09-01 produced in *E. coli*. (1) The concentration of the protein was determined with the Bradford method (1976). (2) The molecular weight of the protein was determined after sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). (3) The purity was estimated by qualitative comparison of the protein bands in the SDS gel. (4) The identity of the protein was confirmed by western blotting. (5) The activity of the protein was determined with the malachite green dye method (Lanzetta *et al.*, 1979) with minor modifications.

3. TEST ITEM AND REFERENCE ITEM

3.1 Test item

The test item was purified protein produced in *Escherichia coli*. The protein was produced at Bayer HealthCare AG (Wuppertal, Germany) according to the purification protocol described in Priestman *et al.* (2005). One aliquot of protein was received. Upon arrival at the Bayer BioScience N.V. test facility, the frozen aliquot was stored in the ultrafreezer (112UF – position 8-03) until the analysis took place.
Protein stored in the ultrafreezer is stable for at least 2 years.
To perform the analysis an aliquot was taken from the test item stock solution. This aliquot received the identification number PR003-01

Test Item ID:	T09-01
Test Item Identity:	2mEPSPS protein
Origin:	*Escherichia coli*
Batch n°:	LEJ5837
Expiry date:	April 3th, 2008
Buffer solution	100 mM Tris (pH 7.5), 2.7 mM KCl, 137 mM NaCl, 1 mM DTT

3.2 Reference items

No reference items were used in this study.

3.3 Standards

The Precision Plus Protein™ Standard Dual Color Marker was supplied by BioRad. The stock solution of the Precision Plus Protein™ Standard Dual Color Marker was stored in freezer 90FZ located in room L79. After opening the Precision Plus Protein™ Standard Dual Color Marker was stored in refrigerator 91RF in room L79.

4. ANALYSIS OF THE TEST ITEM T09-01

The concentration of the protein was estimated and the purity plus the identity of the protein was confirmed on the basis of five techniques. The concentration of the protein was determined according to the method of Bradford (1976). The molecular weight is a distinctive characteristic of a protein and was estimated to confirm the identity by performing a denaturing SDS-PAGE analysis. The same gel was used to estimate the purity of the produced protein. Western blotting is the fourth technique used to confirm the identity of the protein. This technique demonstrates the immunological relationship of the investigated protein to 2mEPSPS proteins by the reactivity of available antibodies directed towards 2mEPSPS. The activity of the purified protein was also assayed and indicates that the protein has the correct enzymatic nature.

4.1 Quantification of the test item T09-01

The Coomassie Plus™ kit (Pierce) is a ready-to-use Bradford assay for protein quantification. When the coomassie dye of the kit binds protein in an acidic medium, an immediate shift in absorption maximum occurs from 465 nm to 595 nm with a concomitant color change from brown to blue. To perform the assay in either test tube or microplate format: a small amount of protein sample is combined with the assay reagent, mixed well, incubated briefly and the absorbance at 595 nm is measured. Protein concentrations are estimated by reference to absorbances obtained from a series of standard protein dilutions, which are assayed alongside the unknown samples.

A dilution series of the test item protein was made to determine the concentration of the protein according to SOP BBS 07/60/00. The dilution series samples were mixed with the assay reagents of the Coomassie Plus™ kit. With the Magellan software, the concentration of the protein samples was estimated by fitting the measured absorbances to a standard curve of known dilutions of bovine serum albumin. A correlation coefficient of 0.99 was obtained for the standard curve. The average of the concentration and the standard deviation was calculated with the Microsoft Excel software (Table 1). The average protein concentration was estimated from the observed three data points. A large standard deviation was introduced by the dilution factor of the sample. A small error in the highest dilution gets multiplied by 400 and becomes much bigger. Therefore we consider the 100 fold dilution to be the most accurate. The 50 fold dilution would give us a smaller deviation but is situated in a less accurate part of the standard curve. The most accurate estimation is 0.93 mg/ml.

4.2 Molecular weight determination of the test item T09-01

SDS-PAGE is an analytical technique used to separate proteins through an acrylamide gel based on their relative molecular weight. The migration distance of a sample is directly proportional to the molecular size of the protein.

The test item protein was separated from side products/protein trace amounts with the SDS-PAGE technique according to SOP BBS 07/63/00 and the obtained gel (041106A, fig. 1) was stained according to SOP BBS 07/66/00.

The logarithm of the molecular weight of the standards was plotted versus the distance of migration with the Microsoft Excel software. A standard curve based on 6 data points was made covering the molecular weight range of the test item. The correlation coefficient of this standard curve was 0.99 (fig. 2). The molecular weight of the test item was extrapolated from the graph by using the equation $y = -0.684x + 3.7023$. The obtained x-value is 4.68. The molecular weight of the test item corresponds to the inverse logarithm of the x-value and is estimated to be 48 kDa. The molecular weight deduced from the amino acid sequence was 47 kDa. Since the error margin of the technique in the molecular weight region of the protein is calculated to be 3 kDa, the deduced molecular weight is confirmed within the limitation of the analysis.

4.3 Purity analysis of test item T09-01

The purity of the test item was estimated by quantification of the bands visible on the SDS gel and calculating the percentage of each band compared to the total density observed.

The purity of the test item was analyzed on the stained protein gel 041106A with the FluorS™ MultiImager according SOP BBS 04/40/00. A representative digitized image was made and an analysis report was prepared. Based on the relative density of each individual band, the purity of the test item was estimated to be 99.52%.

4.4 Electrotransfer of proteins to a membrane

SDS-PAGE was used to separate the proteins through an acrylamide gel in an electrical field based on molecular weight. Afterwards the separated proteins were transferred to a PVDF membrane to make them accessible for the detection with antibodies.

The test item protein was separated from side products/protein trace amounts with the SDS-PAGE technique according to SOP BBS 07/63/00 and the obtained PAGE gel (041106E) was further processed according to SOP BBS 07/64/00. Since the prestained molecular weight markers were visible on the membrane (041106F, fig. 3) we concluded that the transfer to the PVDF membrane was successful.

4.5 Western blotting

Rabbit antiserum anti 2mEPSPS
The rabbit anti 2mEPSPS was produced by and tested at Bayer Bioscience N.V. An aliquot of the antibody was stored in freezer 90FZ located in room L79 until used.

Goat antiserum raised against rabbit IgG coupled with Alkaline Phosphatase
The goat anti-rabbit antiserum coupled with alkaline phosphate was supplied by Sigma. The antibody was stored in the refrigerator 91RF in room L79 until used

The immobilization of proteins to a membrane (041106C) makes them accessible for the reaction with a specific antibody. The membrane (041106C), obtained by electrotransfer of the protein was treated according to SOP BBS 07/65/00.

Protein-free areas on the membrane were blocked overnight by incubation with StartingBlock™ (PBS) Blocking buffer (Pierce). After washing the membrane was incubated with a 1:10000 dilution of rabbit anti 2mEPSPS (Bayer Bioscience NV). After washing the reaction was incubated with a goat anti-rabbit antibody coupled to alkaline phosphatase (1:7000). Addition of substrate caused two bands to become visible. This result is in conflict with the SDS-PAGE data where only one band became visible after staining. The experiment was repeated and a membrane 041106F was generated. After staining only one major band became visible (fig. 3).

4.6 EPSPS activity assay

In the shikimate pathway, phosphoenolpyruvate and erythro-4-phosphate are converted to chorismate through seven enzymatic steps. EPSPS catalyzes the transfer of the enolpyruvyl moiety of PEP to the 5-OH hydroxyl group of shikimate 3-phosphate and releases inorganic phosphate. The EPSPS activity assay was measured according to a colorimetric method described by Forlani et al. (1994) and described in SOP BBS 07/74/00. The inorganic phosphate release is measured using the malachite green dye method (Lanzetta et al., 1979) with minor modifications described in SOP BBS 07/74/00.

Reaction mixtures were made according to SOP BBS 07/74/00 and placed at 37°C for 20 minutes. Reactions were made visible by a colorimetric solution and stopped with the addition of 34% sodiumcitrate after 60 seconds. Samples were transferred to a microtiterplate and the absorbance was read at 660 nm. The absorbance of the duplicates of the buffer control sample was 0.0537 and 0.0536. The absorbance of the duplicates of the test item sample was 0.1562 and 0.1597.

5. RESULTS AND DISCUSSION

The protein concentration of the test item T09-01 was estimated to be 0.93 mg/ml and the purity is estimated to be 99.52%. Based on the migration of the protein in the SDS-PAGE we confirmed the experimental molecular weight of the protein to be comparable to the deduced molecular weight from the amino acid composition, taking into account the error margin of the technique. As expected the test item reacts with an antibody directed towards 2mEPSPS. This reaction demonstrates the immunological relationship of the test item with 2mEPSPS. An activity assay confirmed the activity of the test item.

6. CONCLUSION

Biochemical analyses were performed to confirm the identity of test item T09-01. Based on the analyses we identified the T09-01 test item to be 2mEPSPS. The concentration of the protein in the test item T09-01 was estimated at 0.93 mg/ml with an estimate purity of 99.52%.

7. ARCHIVING

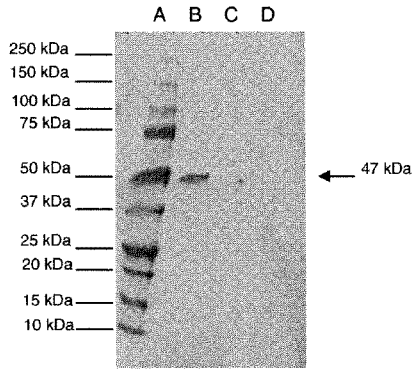
The study plan, the final report and the study data are archived under study number BBS06-003 in the GLP Test Facility Archives of Bayer BioScience N.V., Technologiepark 38, 9052 Gent, Belgium.

A sample of test item (T09-01) was archived in the archive ultrafreezer 113UF of the GLP Test Facility Archives of Bayer BioScience N.V., Technologiepark 38, 9052 Gent, Belgium (room number L57).

Table 1: Protein concentration of test item T09-01

Subsample	Dilution factor	Measured concentration µg/ml	Concentration of test item mg/ml
PR003-1A	400	4.54	1.82
PR003-1B	100	9.31	0.93
PR003-1C	50	14.74	0.74

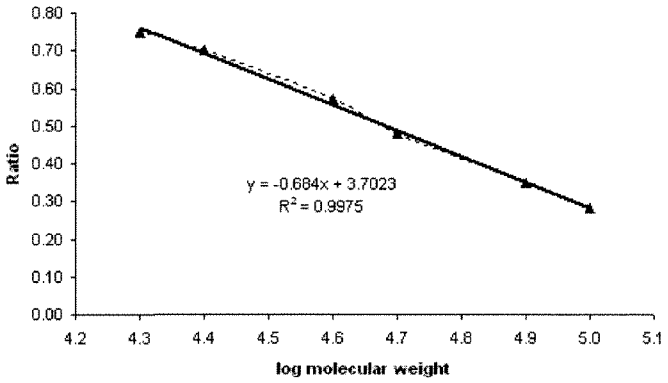
Figure3: Western blot of test item T09-01 (blot ID# 041106F)



Legend : A molecular weight marker
B 0.6 µg test item T09-01
C 0.3 µg test item T09-01
D 0.1 µg test item T09-01

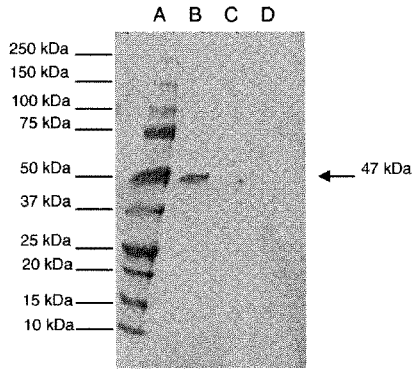
Figure 2: Plot standard curve molecular weight of test item T09-01

A	B	C	D	E
Molecular weight kDa	log Molecular weight	Migrated distance cm	Total distance cm	Ratio C/D
100	5.0	1.7	6.0	0.28
75	4.9	2.1	6.0	0.35
50	4.7	2.9	6.0	0.48
37	4.6	3.4	6.0	0.57
25	4.4	4.2	6.0	0.70
20	4.3	4.5	6.0	0.75



Legend : Plot of the logarithm of molecular weight of the standard proteins plotted against the ratio of the migrated distance and the total distance.

Figure3: Western blot of test item T09-01 (blot ID# 041106F)



Legend : A molecular weight marker
B 0.6 µg test item T09-01
C 0.3 µg test item T09-01
D 0.1 µg test item T09-01

REFERENCES

No	Doc No	Author(s), year, title, source, edition, pages
1.	M-268549-01-1	Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. Analytical Biochemistry 72, 248-254
2.	M-268612-01-1	Lanzetta P.A., Alvarez L.J., Reinach P.S. and Candia O.A. (1979) An improved assay for nanomole amounts of inorganic phosphate. Analytical Biochemistry 100, 95-97
3.	M-269790-01-1	Priestman M.A., Funke T., Singh I.M., Crupper S.S. and Schonbrunn E. (2005) 5-Enolpyruvylshikimate-3-phosphate synthase from Staphylococcus aureus is sensitive to glyphosate. FEBS Letters 579, 728-732
4.	M-268551-01-1	Forlani G., Parisi B. and Nielsen E. (1994) 5-enol-Pyruvyl-Shikimate-3-Phosphate Synthase from Zea mays cultured cells. Plant Physiol. 105, 1107-1114

Study data index

Bayer BioScience N.V. Study number: BBS06-003

Study Title: Certificate of analysis for the 2mEPSPS protein produced in *E. coli*

Total number of study data pages: 71 pages

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Pepsin from porcine gastric mucosa, lyophilized powder, 3,200-4,500 units/mg protein
Product Number	P6887
Product Brand	Sigma-Aldrich
CAS Number	9001-75-6
Storage Temp	−20°C

TEST	SPECIFICATION	LOT 074K77164 RESULTS
APPEARANCE	REPORT RESULT	OFF-WHITE LYOPHILIZED POWDER
PROTEIN BY UV ABSORBANCE	REPORT RESULT	100%
ENZYMATIC ACTIVITY	3,200 TO 4,500 UNITS/MG PROTEIN	3,300 UNITS/MG PROTEIN
UNIT DEFINITION	ONE UNIT WILL PRODUCE A CHANGE IN A280 OF 0.001 PER MINUTE AT PH2.0 AT 37DEGC, MEASURED AS TCA-SOLUBLE PRODUCTS USING HEMOGLOBIN AS SUBSTRATE. (FINAL VOLUME = 16ML. LIGHT PATH = 1CM.)	
SHELF LIFE	2 YEARS	NOVEMBER 2006
QC ACCEPTANCE DATE		NOVEMBER 2004

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Peroxidase from Horseradish, Type VI-A, ~1000 units/mg solid (using ABTS), 250-330 units/mg solid (using pyrogallol), essentially salt-free, lyophilized powder
Product Number	P6782
Product Brand	Sigma
CAS Number	9003-99-0
Molecular Formula	
Molecular Weight	

TEST	SPECIFICATION	LOT 111K74351 RESULTS
APPEARANCE	RED-BROWN POWDER	CONFORMS
SOLUBILITY	CLEAR AMBER TO AMBER-BROWN SOLUTION AT 10 MG PLUS 1 ML OF 0.1 M PHOSPHATE BUFFER, PH 6.0	CONFORMS
ENZYMATIC ACTIVITY	APPROX. 1000 UNITS PER MG SOLID (USING ABTS)	987 UNITS/MG SOLID
ENZYMATIC ACTIVITY	250 TO 330 UNITS/MG SOLID (USING PYROGALLOL)	288 UNITS/MG SOLID
RZ	APPROX. 3.0. THE RZ IS DEFINED AS THE RATIO OF THE ABSORBANCE AT 403NM TO THE ABSORBANCE AT 275NM. THIS VALUE IS AN EXPRESSION OF THE RATIO OF HEMIN TO PROTEIN CONTENT.	RZ: 2.7
SHELF LIFE SOP QC-12-006	5 YEARS	JANUARY 2007
QC ACCEPTANCE DATE		JANUARY 2002

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Albumin from chicken egg white, Grade V, ≥98% (agarose gel electrophoresis), lyophilized powder
Product Number	A5503
Product Brand	Sigma
CAS Number	9006-59-1
Molecular Formula	
Molecular Weight	

TEST	SPECIFICATION	LOT 031K7025 RESULTS
APPEARANCE	WHITE TO YELLOW POWDER	WHITE POWDER
SOLUBILITY	CLEAR TO SLIGHTLY HAZY COLORLESS TO YELLOW SOLUTION AT 40MG/ML IN WATER	SLIGHTLY HAZY COLORLESS
WATER BY KARL FISCHER	NMT 6%	5%
ELEMENTAL ANALYSIS	13.0 TO 16.0% NITROGEN	14.2%
AGAROSE ELECTROPHORESIS	MINIMUM 98%	>99%
SHELF LIFE SOP QC-12-006	5 YEARS	JUNE 2006
QC ACCEPTANCE DATE		JUNE 2001

Lori Schulz, Manager
Analytical Services
St. Louis, Missouri USA

ATTACHMENT 3 - **GLP COMPLIANCE CERTIFICATES (AND ENGLISH TRANSLATION)**

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

GROUPE INTERMINISTERIEL DES PRODUITS CHIMIQUES

Paris, le 13 DEC. 2005

OBJET : Evaluation de la conformité aux B.P.L. selon la directive 2004/10/CE du 11 février 2004.

Consécutivement à votre engagement vis à vis du GIPC et du COFRAC et en application du décret n° 81-278 du 25 mars 1981 portant création d'un Groupe Interministériel des Produits Chimiques (GIPC), modifié notamment par le décret 90-206 du 7 mars 1990 et par le décret n° 98-1312 du 31 décembre 1998 concernant les bonnes pratiques de laboratoires, je vous confirme que le GIPC, au vu des résultats du contrôle exercé par le Comité français d'accréditation (COFRAC) - Section Laboratoires a décidé pour votre installation du statut suivant :

Respect des principes de B.P.L.
Domaines de reconnaissance :

- ☐ **2 - études de toxicité**
- ☐ **3 - études de mutagénicité**
- ☐ **4 - études écotoxicologiques sur les organismes aquatiques et terrestres**
- ☐ **8 - méthodes de chimie analytique et clinique**
- ☐ **9 - autres études (métabolisme animal)**

Date d'inspection : 26 & 27 octobre 2005 ☐ **inspection de renouvellement (i.r)**

Date de décision du G.I.P.C. : 9 décembre 2005
Date de prise d'effet : 27 octobre 2005
Année de première conformité : 1992
Durée de validité : 18 mois

P. O.
Le Président,

Pierre CREYSSEL
Conseiller d'Etat h.

BAYER CROPSCIENCE
355 rue Dostoievski
BP 153
06903 Sophia Antipolis Cedex

Secrétariat général du GIPC - DGE- Simap - 12, rue Villiot - 75572 Paris cedex 12
Téléphone : 01 53 44 96 10 – Télécopie : 01 53 44 91 72

REPUBLIC OF FRANCE

INTERMINISTERIAL GROUP FOR CHEMICAL PRODUCTS

Paris, the 13 Dec. 2005

SUBJECT: Evaluation of G.L.P conformity to directive 2004/10/CE of 11 February 2004

Following your commitment vis-a-vis GIPC and COFRAC and by applying for decree n°. 81-278 of 25 March 1981, supporting the creation of an Interministerial Group for Chemical Products (GIPC), modified, among others, by decree 90-206 of 7th March, 1990 and by decree n° 98-1312 of 31 December 1998 concerning good laboratory practice, I confirm that the GIPC, in view of the results of the inspection carried out by the French Accreditation Committee (COFRAC) - Tests Section has decided on the following status for your test installation:

Observing the G.L.P principles

Areas of recognition

☐ **2 - toxicity studies**
☐ **3 - mutagenicity studies**
☐ **4 - ecotoxicology studies on aquatic and terrestrial organisms**
☐ **8 - analytical and clinical chemistry methods**
☐ **9 - other studies (animal metabolism)**

Inspection date: 26 & 27 October 2005 ☐ renewal inspection (i.r)

Date of GIPC decision: 9 December 2005
Date of taking effect: 27 October 2005
Year of first conformity: 1992
Validity duration: 18 months

The President
Pierre CREYSSEL
State Advisor

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