Bayer CropScience

2mEPSPS protein

ACUTE TOXICITY

BY INTRAVENOUS INJECTION IN MICE

DATA REQUIREMENTS
Based on the U.S. E.P.A. Health Effects Test Guidelines OPPTS 870.1100 adopted in 2002 and on the O.E.C.D. Test Guideline 425 adopted in 2001

REPORT OF STUDY SA 07014
Sponsor identification number: Lynx-PSI N° TX99X110

AUTHOR / STUDY DIRECTOR: D. ROUQUIE

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STUDY COMPLETED ON: FEBRUARY 07, 2008

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 § 10 (d) (1) (A), (B) or (C).

Company Name:

Company Agent:

Title:

Signature:

Date: _____

These data are the property of Bayer CropScience, and as such, are considered to be 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 other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study here reported was not performed in compliance with the principles of Good Laboratory Practice ("Bonnes Pratiques de Laboratoire") in that it was not subjected to specific Quality Assurance inspections. It was performed according to the standard operating procedures which were previously accepted and periodically inspected by the Quality Assurance Unit.

Author / Study Director:

Date: February 04, 2008

D. ROUQUIE

Sponsor Representative:

Date: 06 FEBRUARY 2008

C. HERQUET-GUICHENEY

Study Submitter:

Date:

FLAGGING STATEMENTS

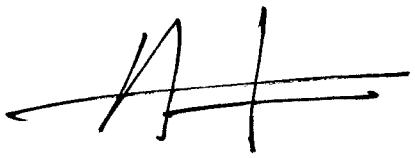
This page is reserved for flagging statements as may be required by U.S. E.P.A.

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:



Date: February 04, 2008

D. ROUQUIE

STUDY PROFESSIONALS

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

STUDY DIRECTOR	:	D. ROUQUIE
LABORATORY ANIMAL RESOURCES	:	J.P. KOCWIN
TOXICOLOGY SUPERVISOR	:	D. SANTAMARIA
RESPONSIBLE TECHNICIAN	:	S. LEBAS
REPORT UNIT ASSISTANT	:	P. ALMERAS

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SUMMARY

This study was based on the U.S. E.P.A. Health Effects Test Guidelines OPPTS 870.1100 adopted in 2002 and on the O.E.C.D. Test Guideline 425 adopted in 2001.

The objective was to assess the acute intravenous toxicity of 2mEPSPS protein in OF1 mice. Aprotinin and melittin were served as negative and positive control, respectively and were also tested in this study.

Groups of 5 female OF1 mice were administered either with the 2mEPSPS protein, or the aprotinin or the melittin proteins in PBS buffer by intravenous injection at dose levels of 1 and 10 mg/kg body weight. A group of 5 female mice received only the vehicle and acted as a control. All animals were observed for clinical signs daily for fifteen days whilst their body weights were measured weekly. At termination of the study period, animals were subjected to a necropsy including macroscopic examination.

As expected, all the animals of the positive control group (melittin at 10 mg/kg body weight) died on the day of treatment. Animals treated at 1 mg/kg of melittin and negative control animals treated with aprotinin at 1 or 10 mg/kg body weight or with the vehicle only showed no visible signs of systemic toxicity.

There were no mortalities or treatment-related toxic effects in female OF1 mice after an acute intravenous administration of the 2mEPSPS protein at 1 or 10 mg/kg body weight.

In conclusion, under the conditions of this study, the 2mEPSPS protein was found to be non acutely toxic by the intravenous route.

INTRODUCTION

The objective was to assess the potential for acute toxicity of the 2mEPSPS protein when administered intravenously at dose levels of 1 and 10 mg/kg body weight in the OF1 mouse. In addition, the acute intravenous toxicity of aprotinin (negative control) and melittin (negative control at 1 mg/kg body weight and positive control at 10 mg/kg body weight) were also compared.

This study was based on the U.S. E.P.A. Health Effects Test Guidelines OPPTS 870.1100 adopted in 2002 and on the O.E.C.D. Test Guideline 425 adopted in 2001.

The study protocol is presented in Attachment 1.

The study time schedule was as follows:

Study initiation date *	February 26, 2007
Animal arrival date	February 28, 2007
Experimental starting date	February 28, 2007
Sponsor protocol approval date	February 26, 2007
Randomization date (Day -1)	March 06, 2007
Dosing date	March 07, 2007
Final sacrifice date	March 21, 2007
Experimental completion date	March 21, 2007

* Date of protocol approval by Study Director.

MATERIAL AND METHODS

1 - CONTROLS AND TEST SUBSTANCE FORMULATIONS

The test substance 2mEPSPS protein was used in this study. Information on the chemical characterization of the test substance was documented by Bayer Bioscience NV, B-9052 Ghent, Belgium and is presented in Attachment 2. The test substance was stored in an air-tight, light resistant container at approximately -20°C.

The aprotinin protein, a serin protease inhibitor (molecular weight of approximately 6 512 Da, reference number A4529, batch number 080K7022) was used as a negative control. Chemical information on aprotinin was documented by Sigma-Aldrich and is presented in Attachment 2. This substance was stored in an air-tight, light-resistant container at approximately +4°C.

The melittin protein, the principle hemolytic component of honey bee venom (molecular weight of approximately 2 847 Da, reference number M2272, batch number 021K4000) was used as negative (at 1 mg/kg body weight) and positive protein controls (at 10 mg/kg body weight). Chemical information on melittin was documented by Sigma-Aldrich and is presented in Attachment 2. This substance was stored in an air-tight, light-resistant container at approximately -20°C.

The formulations were prepared by dissolving the substances in PBS buffer (13.7 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.3) to produce the required concentration (w/v or v/v). Therefore, a vehicle control was also included in the study design.

The formulations were placed in air-tight glass bottles at room temperature and were used as quickly as practicable after preparation.

2 - ANIMALS, HOUSING, DIET AND WATER

2.1 Animals

The mouse was chosen because of it is recommended by regulatory authorities as an appropriate test species to assess acute toxicity. The Crl:OF1 strain was used since sufficient background toxicity data exist to support interpretation of results. A total of 40 female Crl:OF1 mice were obtained from Charles River Laboratories, Saint Germain sur l'Arbresle, France. Animals were acclimatized to laboratory conditions for 6 days prior to treatment and were 7 weeks old at the start of treatment.

a/Selection and randomization

All animals were examined for mortality and clinical signs during the acclimatization phase. Two days before test substance administration, all animals were weighed. Five female mice per group were selected for the study. An automatic randomization procedure was used to select animals for the study from the middle of the weight range of the available animals ensuring body weights were within ± 20% of the mean body weight at randomization. Selected animals were in a weight range from 23.2 to 28.0 g on the day of treatment.

b/Identification

One day before treatment following randomization, animals were assigned permanent identification numbers within groups. Each animal was identified by a stainless steel ear tag bearing a unique animal number.

2.2 Housing

Mice were housed individually in suspended, stainless steel, wire-mesh cages. The temperature, humidity and lighting in the animal room were constantly monitored by an automatic system.

The target specifications were:

- temperature: 20°C- 24°C
- humidity: 40-70%
- lighting: 12-hour light, 12-hour dark cycles (7 am - 7 pm)

The ventilation system in the animal room was maintained to ensure adequate ventilation, with the performance of the system regularly checked for a target specification of 10 to 15 air changes per hour.

There were no deviations from target specifications which could have compromised the study. Housing data are placed in the study file.

2.3 Diet and water

Certified rodent pelleted and irradiated diet A04C-10 from S.A.F.E. (Scientific Animals Food and Engineering, Augy, France) was available *ad libitum*. Filtered and softened tap water from the municipal water supply was provided *ad libitum* using water bottles. Filters servicing the watering system were regularly changed and sterilization of the system was periodically performed. Certificates of analysis were provided by the diet manufacturer and the supplier. Additionally, quality control analytical reports of the physicochemical properties and concentration of specified contaminants were periodically obtained from independent consultant analysts. These routine analyses of feed and water indicated that there was no contamination which could have affected the integrity and outcome of this study.

3 - EXPERIMENTAL DESIGN

Groups of 5 female mice were given a single intravenous injection of the test or control substances. The test proteins were administered in solution in PBS buffer at dose levels of 1 or 10 mg/kg body weight, intravenously through the tail vein at a volume of 10 ml/kg (based on body weight on Day 1). The high dose was chosen based on the limit of solubility of the test material.

Details of group sizes and treatments:

GROUP	CONTROL OR TEST SUBSTANCE(S)	DOSE LEVELS mg/kg	NUMBER OF FEMALE ANIMALS PER GROUP	ANIMALS IDENTITY FEMALES
1	Vehicle control	0	5	RT1F0482 to 0486
2	Aprotinin	1	5	RT2F0487 to 0491
3		10	5	RT3F0492 to 0496
4	Melittin	1	5	RT4F0497 to 0501
5		10	5	RT5F0502 to 0506
6	Test substance: 2mEPSPS protein	1	5	RT6F0507 to 0511
7		10	5	RT7F0512 to 0516

Clinical signs were recorded daily from Day 1 through Day 15. They were recorded approximately 30 minutes after each dosing, at least once more on Day 1 and at least once each day thereafter. The nature, onset, severity, reversibility and duration of all clinical signs were recorded. Cages and cage-trays were inspected daily for evidence of ill-health, such as blood or loose feces. In addition, animals were checked twice daily for mortality, except on weekends and public holidays when they were checked once daily.

4 - BODY WEIGHT

Each animal was weighed on Days -5, -1, 1, 8 and 15.

5 - POST MORTEM PROCEDURES

At final sacrifice on Day 15, surviving animals were anesthetized by Isoflurane inhalation (Baxter, Maurepas, France), then exsanguinated under deep anesthesia before necropsy. Necropsy included macroscopic examination of abdominal and thoracic cavities, major organs and tissues. Significant macroscopic abnormalities were recorded.

6 - CALCULATIONS

Means and standard deviations were calculated for body weights and absolute body weight gains.

7 - DATA STORAGE

All raw data, supporting documents, as well as protocol, protocol amendments and final report are maintained in the document archive room. A test substance reference sample is retained in the area of the products storeroom defined for the archiving of test substances. 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

1 - MORTALITY (Tab. 1)

Group mortality:

Groups	Control or Test substances	Dose levels (mg/kg)	Number of dead female animals
1	Vehicle control	0	0 / 5
2	Aprotinin	1	0 / 5
3		10	0 / 5
4	Melittin	1	0 / 5
5		10	5 / 5
6	Test substance:	1	0 / 5
7	2mEPSPS protein	10	0 / 5

No mortality was observed after administration of the 2mEPSPS protein.

All mice treated with 10 mg/kg of melittin died within 4 hours after intravenous injection on Day 1. These results are in agreement with the LD50 for melittin which is approximately 3 mg/kg (1). As expected, no mortality was observed during the study in melittin treated animals at 1 mg/kg, or in negative controls treated at 1 or 10 mg/kg aprotinin.

2 - DAILY OBSERVATIONS (Tab. 1)

No clinical signs were observed in 2mEPSPS protein-treated animals and in negative control groups (melittin at 1 mg/kg and aprotinin at both doses) throughout the study period.

3 - BODY WEIGHT (Tab. 2, 3)

Mean body weight gain over the entire study was similar between groups of animals that received the 2mEPSPS protein and the negative control protein.

Since no changes were observed in term of body weight gain between negative control protein and 2mEPSPS protein groups, it can be concluded that there is no adverse effect on body weight gain following treatment with the 2mEPSPS protein.

4 - GROSS PATHOLOGY (Tab. 4)

No macroscopic observations were observed in all the animals examined and in particular in the 2mEPSPS protein-treated animals.

Therefore, no 2mEPSPS protein -treatment related macroscopic findings were observed.

CONCLUSION

As expected, all the animals of the positive control group (melittin at 10 mg/kg body weight) died on the day of treatment, demonstrating the sensitivity of the test system. Animals treated at 1 mg/kg of melittin and negative control animals treated with aprotinin at 1 or 10 mg/kg body weight or with the vehicle only showed no visible signs of systemic toxicity.

There were no mortalities and no treatment-related toxic effects in female OF1 mice after an acute intravenous administration of the 2mEPSPS protein at 1 or 10 mg/kg body weight. In conclusion, under the conditions of the study, the 2mEPSPS protein was found to be non acutely toxic by the intravenous route.

REFERENCE

1 - SCHMIDT J.O., (1995): Toxinology of venoms from the honeybee genus Apis. Toxicon., 33, pp. 917-927.

PROTOCOL DEVIATIONS

There were no protocol deviations during the study.

Author / Study Director:

Date: February 07, 2008

D. ROUQUIE

ABBREVIATIONS

2mEPSPS	Double mutated maize 5 enol pyruvylshikimate-3-phosphate synthase
%	Percentage
°C	Degree Celsius
am	*ante meridiem*
Da	Daltons
E.E.C.	European Economic Communities
E.P.A.	Environmental Protection Agency
g	Gram
GLP	Good Laboratory Practice
LD_{50}	Lethal Dose, 50%
M.A.F.F.	Ministry of Agriculture, Forestry and Fisheries
mg/kg	Milligram/kilogram
ml/kg	Milliliter/kilogram
mM	Millimolar
O.E.C.D.	Organization for Economic Cooperation and Development
pm	*post meridiem*
QA	Quality Assurance
SDEVS	Standard Deviation
Tab.	Table
U.S.	United States
USA	United States of America
v/v	Volume/volume
w/v	Weight/volume

TABLES

TABLE 1 - **INDIVIDUAL CLINICAL SIGNS AND DEAD ANIMAL STATUS**

GROUP	CONTROL OR TEST SUBSTANCE(S)	DOSE LEVELS mg/kg	NUMBER OF FEMALE ANIMALS PER GROUP	ANIMALS IDENTITY FEMALES
1	Vehicle control	0	5	RT1F0482 to 0486
2	Aprotinin	1	5	RT2F0487 to 0491
3		10	5	RT3F0492 to 0496
4	Melittin	1	5	RT4F0497 to 0501
5		10	5	RT5F0502 to 0506
6	Test substance: 2mEPSPS protein	1	5	RT6F0507 to 0511
7		10	5	RT7F0512 to 0516

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INDIVIDUAL CLINICAL SIGNS TABLE
Study number: SA 07014
DATES 07-Mar-07 TO 21-Mar-07
Study start date: 07-Mar-07

Printed: 08-Oct-07
Page: 1
Acute Toxicity/Intravenous screen

DOSAGE LEVEL IN: mg/kg

ANIMAL		OBSERVATION	DAYS OBSERVED
RT1F0482	0.0	NORMAL THROUGHOUT INTERVAL	
RT1F0483	0.0	NORMAL THROUGHOUT INTERVAL	
RT1F0484	0.0	NORMAL THROUGHOUT INTERVAL	
RT1F0485	0.0	NORMAL THROUGHOUT INTERVAL	
RT1F0486	0.0	NORMAL THROUGHOUT INTERVAL	
RT2F0487	1.0	NORMAL THROUGHOUT INTERVAL	
RT2F0488	1.0	NORMAL THROUGHOUT INTERVAL	
RT2F0489	1.0	NORMAL THROUGHOUT INTERVAL	
RT2F0490	1.0	NORMAL THROUGHOUT INTERVAL	
RT2F0491	1.0	NORMAL THROUGHOUT INTERVAL	
RT3F0492	10.0	NORMAL THROUGHOUT INTERVAL	
RT3F0493	10.0	NORMAL THROUGHOUT INTERVAL	
RT3F0494	10.0	NORMAL THROUGHOUT INTERVAL	
RT3F0495	10.0	NORMAL THROUGHOUT INTERVAL	
RT3F0496	10.0	NORMAL THROUGHOUT INTERVAL	
RT4F0497	1.0	NORMAL THROUGHOUT INTERVAL	
RT4F0498	1.0	NORMAL THROUGHOUT INTERVAL	
RT4F0499	1.0	NORMAL THROUGHOUT INTERVAL	
RT4F0500	1.0	NORMAL THROUGHOUT INTERVAL	
RT4F0501	1.0	NORMAL THROUGHOUT INTERVAL	
RT5F0505	10.0	NORMAL THROUGHOUT INTERVAL	
RT6F0507	1.0	NORMAL THROUGHOUT INTERVAL	
RT6F0508	1.0	NORMAL THROUGHOUT INTERVAL	

DOSAGE LEVEL IN: mg/kg

ANIMAL		OBSERVATION	DAYS OBSERVED
RT6F0509	1.0	NORMAL THROUGHOUT INTERVAL	
RT6F0510	1.0	NORMAL THROUGHOUT INTERVAL	
RT6F0511	1.0	NORMAL THROUGHOUT INTERVAL	
RT7F0512	10.0	NORMAL THROUGHOUT INTERVAL	
RT7F0513	10.0	NORMAL THROUGHOUT INTERVAL	
RT7F0514	10.0	NORMAL THROUGHOUT INTERVAL	
RT7F0515	10.0	NORMAL THROUGHOUT INTERVAL	
RT7F0516	10.0	NORMAL THROUGHOUT INTERVAL	

NOTE: DATA FOR Dosing phase 1

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Sophia-Antipolis
Mouse/OF 1

Dead Animal Status List for All Animals
Study number: SA 07014
Study start date: 07-Mar-07

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Acute Toxicity/Intravenous screen

Animal Number	Grp	Sex	Study Phase	Date and Time Data was Entered	Oper. No.	Date of Death	Phase Day	Death Typ	Death Status	Term. Body Wt. (g)	Ow	Grs
RT1F0482	1	F	Dosing phase 1	21-Mar-07 10:17	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT1F0483	1	F	Dosing phase 1	21-Mar-07 10:17	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT1F0484	1	F	Dosing phase 1	21-Mar-07 10:17	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT1F0485	1	F	Dosing phase 1	21-Mar-07 10:18	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT1F0486	1	F	Dosing phase 1	21-Mar-07 10:18	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT2F0487	2	F	Dosing phase 1	21-Mar-07 10:18	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT2F0488	2	F	Dosing phase 1	21-Mar-07 10:19	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT2F0489	2	F	Dosing phase 1	21-Mar-07 10:19	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT2F0490	2	F	Dosing phase 1	21-Mar-07 10:19	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT2F0491	2	F	Dosing phase 1	21-Mar-07 10:20	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT3F0492	3	F	Dosing phase 1	21-Mar-07 10:20	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT3F0493	3	F	Dosing phase 1	21-Mar-07 10:20	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT3F0494	3	F	Dosing phase 1	21-Mar-07 10:20	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT3F0495	3	F	Dosing phase 1	21-Mar-07 10:21	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT3F0496	3	F	Dosing phase 1	21-Mar-07 10:21	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT4F0497	4	F	Dosing phase 1	21-Mar-07 10:21	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT4F0498	4	F	Dosing phase 1	21-Mar-07 10:22	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT4F0499	4	F	Dosing phase 1	21-Mar-07 10:23	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	-
RT4F0500	4	F	Dosing phase 1	21-Mar-07 10:23	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT4F0501	4	F	Dosing phase 1	21-Mar-07 10:24	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT5F0502	5	F	Dosing phase 1	07-Mar-07 10:55	57	07-Mar-07	1	u	Found dead	-----	-	C
RT5F0503	5	F	Dosing phase 1	07-Mar-07 10:55	57	07-Mar-07	1	u	Found dead	-----	-	C
RT5F0504	5	F	Dosing phase 1	07-Mar-07 10:55	57	07-Mar-07	1	u	Found dead	-----	-	C
RT5F0505	5	F	Dosing phase 1	07-Mar-07 15:25	57	07-Mar-07	1	u	Found dead	-----	-	C
RT5F0506	5	F	Dosing phase 1	07-Mar-07 10:56	57	07-Mar-07	1	u	Found dead	-----	-	C
RT6F0507	6	F	Dosing phase 1	21-Mar-07 10:24	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT6F0508	6	F	Dosing phase 1	21-Mar-07 10:24	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT6F0509	6	F	Dosing phase 1	21-Mar-07 10:25	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT6F0510	6	F	Dosing phase 1	21-Mar-07 10:25	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT6F0511	6	F	Dosing phase 1	21-Mar-07 10:25	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT7F0512	7	F	Dosing phase 1	21-Mar-07 10:25	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT7F0513	7	F	Dosing phase 1	21-Mar-07 10:26	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT7F0514	7	F	Dosing phase 1	21-Mar-07 10:26	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT7F0515	7	F	Dosing phase 1	21-Mar-07 10:26	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C
RT7F0516	7	F	Dosing phase 1	21-Mar-07 10:27	57	21-Mar-07	15	s	Final phase sacrifice	-----	-	C

Note: * = pretest animal no. P = partial data. C = complete data. - = no data.

TABLE 2 - **MEAN AND INDIVIDUAL BODY WEIGHTS**

GROUP	CONTROL OR TEST SUBSTANCE(S)	DOSE LEVELS mg/kg	NUMBER OF FEMALE ANIMALS PER GROUP	ANIMALS IDENTITY FEMALES
1	Vehicle control	0	5	RT1F0482 to 0486
2	Aprotinin	1	5	RT2F0487 to 0491
3		10	5	RT3F0492 to 0496
4	Melittin	1	5	RT4F0497 to 0501
5		10	5	RT5F0502 to 0506
6	Test substance: 2mEPSPS protein	1	5	RT6F0507 to 0511
7		10	5	RT7F0512 to 0516

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Mouse/OF 1

ANIMAL BODY WEIGHTS IN (G)
Study number: SA 07014
Study start date: 07-Mar-07

Printed: 08-Oct-07
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Acute Toxicity/Intravenous screen

DOSAGE IN mg/kg / ANIMAL		SEX	DAY OF PHASE 1	DAY OF PHASE 8	DAY OF PHASE 15
			FEMALE ANIMALS		
RT1F0482	0.0	F	24.9	24.7	27.0
RT1F0483			24.2	26.8	30.1
RT1F0484			25.2	26.8	29.6
RT1F0485			26.6	29.3	29.9
RT1F0486			24.9	25.7	30.0
		(n)	5	5	5
		MEANS	25.2	26.7	29.3
		SDEVS	0.9	1.7	1.3
RT2F0487	1.0	F	26.0	27.8	31.0
RT2F0488			25.7	26.2	27.8
RT2F0489			27.2	27.8	28.7
RT2F0490			25.3	26.8	30.0
RT2F0491			23.5	26.7	31.3
		(n)	5	5	5
		MEANS	25.5	27.1	29.8
		SDEVS	1.3	0.7	1.5
RT3F0492	10.0	F	25.9	26.2	27.3
RT3F0493			25.2	25.7	27.5
RT3F0494			24.5	25.0	27.1
RT3F0495			27.3	26.9	30.9
RT3F0496			26.4	25.8	30.5
		(n)	5	5	5
		MEANS	25.9	25.9	28.7
		SDEVS	1.1	0.7	1.9
RT4F0497	1.0	F	25.2	27.2	29.2
RT4F0498			25.3	27.5	29.4
RT4F0499			28.0	27.7	30.9
RT4F0500			24.8	26.6	29.1
RT4F0501			23.2	25.7	27.6
		(n)	5	5	5
		MEANS	25.3	26.9	29.2
		SDEVS	1.7	0.8	1.2
RT5F0502	10.0	F	24.8		
RT5F0503			26.1		
RT5F0504			23.9		
RT5F0505			24.7		

NOTE: DATA FOR Dosing phase 1

DOSAGE IN mg/kg			DAY OF PHASE		
ANIMAL		SEX	1	8	15
			FEMALE ANIMALS		
RT5F0506	10.0	F	27.5		
		(n)	5		
		MEANS	25.4		
		SDEVS	1.4		
RT6F0507	1.0	F	25.4	27.5	27.2
RT6F0508			26.8	28.6	32.3
RT6F0509			25.0	26.0	27.9
RT6F0510			25.8	26.2	28.3
RT6F0511			26.5	28.4	28.8
		(n)	5	5	5
		MEANS	25.9	27.3	28.9
		SDEVS	0.7	1.2	2.0
RT7F0512	10.0	F	27.0	28.8	31.5
RT7F0513			26.7	26.5	28.7
RT7F0514			25.7	26.7	27.3
RT7F0515			23.2	25.8	26.9
RT7F0516			25.0	24.5	28.2
		(n)	5	5	5
		MEANS	25.5	26.5	28.5
		SDEVS	1.5	1.6	1.8

NOTE: DATA FOR Dosing phase 1

TABLE 3 - **MEAN AND INDIVIDUAL ABSOLUTE WEIGHT GAINS**

GROUP	CONTROL OR TEST SUBSTANCE(S)	DOSE LEVELS mg/kg	NUMBER OF FEMALE ANIMALS PER GROUP	ANIMALS IDENTITY FEMALES
1	Vehicle control	0	5	RT1F0482 to 0486
2	Aprotinin	1	5	RT2F0487 to 0491
3		10	5	RT3F0492 to 0496
4	Melittin	1	5	RT4F0497 to 0501
5		10	5	RT5F0502 to 0506
6	Test substance: 2mEPSPS protein	1	5	RT6F0507 to 0511
7		10	5	RT7F0512 to 0516

Bayer CropScience
Center of Toxicology
Sophia-Antipolis
Mouse/OF 1

ANIMAL ABSOLUTE WEIGHT GAINS IN (G)
Study number: SA 07014
ABSOLUTE WEIGHT GAINS REFERENCED TO Dosing phase 1 (DAY 1)
Study start date: 07-Mar-07

Printed: 08-Oct-07
Page: 1
Acute Toxicity/Intravenous screen

DOSAGE IN mg/kg / ANIMAL		SEX	DAY OF PHASE 8	DAY OF PHASE 15
			FEMALE ANIMALS	
RT1F0482	0.0	F	-0.2	2.1
RT1F0483			2.6	5.9
RT1F0484			1.6	4.4
RT1F0485			2.7	3.3
RT1F0486			0.8	5.1
		(n)	5	5
		MEANS	1.5	4.2
		SDEVS	1.2	1.5
RT2F0487	1.0	F	1.8	5.0
RT2F0488			0.5	2.1
RT2F0489			0.6	1.5
RT2F0490			1.5	4.7
RT2F0491			3.2	7.8
		(n)	5	5
		MEANS	1.5	4.2
		SDEVS	1.1	2.5
RT3F0492	10.0	F	0.3	1.4
RT3F0493			0.5	2.3
RT3F0494			0.5	2.6
RT3F0495			-0.4	3.6
RT3F0496			-0.6	4.1
		(n)	5	5
		MEANS	0.1	2.8
		SDEVS	0.5	1.1
RT4F0497	1.0	F	2.0	4.0
RT4F0498			2.2	4.1
RT4F0499			-0.3	2.9
RT4F0500			1.8	4.3
RT4F0501			2.5	4.4
		(n)	5	5
		MEANS	1.6	3.9
		SDEVS	1.1	0.6
RT5F0502	10.0	F		
RT5F0503				
RT5F0504				
RT5F0505				

NOTE: DATA FOR Dosing phase 1

DOSAGE IN mg/kg			DAY OF PHASE	
ANIMAL		SEX	8	15
			FEMALE ANIMALS	
RT5F0506	10.0	F		
RT6F0507	1.0	F	2.1	1.8
RT6F0508			1.8	5.5
RT6F0509			1.0	2.9
RT6F0510			0.4	2.5
RT6F0511			1.9	2.3
		(n)	5	5
		MEANS	1.4	3.0
		SDEVS	0.7	1.5
RT7F0512	10.0	F	1.8	4.5
RT7F0513			-0.2	2.0
RT7F0514			1.0	1.6
RT7F0515			2.6	3.7
RT7F0516			-0.5	3.2
		(n)	5	5
		MEANS	0.9	3.0
		SDEVS	1.3	1.2

NOTE: DATA FOR Dosing phase 1

TABLE 4 - **INDIVIDUAL GROSS PATHOLOGY FINDINGS**

GROUP	CONTROL OR TEST SUBSTANCE(S)	DOSE LEVELS mg/kg	NUMBER OF FEMALE ANIMALS PER GROUP	ANIMALS IDENTITY FEMALES
1	Vehicle control	0	5	RT1F0482 to 0486
2	Aprotinin	1	5	RT2F0487 to 0491
3		10	5	RT3F0492 to 0496
4	Melittin	1	5	RT4F0497 to 0501
5		10	5	RT5F0502 to 0506
6	Test substance: 2mEPSPS protein	1	5	RT6F0507 to 0511
7		10	5	RT7F0512 to 0516

Bayer CropScience
Center of Toxicology
Sophia-Antipolis
Mouse/OF 1

Raw Data Listing of Gross Observations with Modifiers and comments
Study number: SA 07014
Study start date: 07-Mar-07

Printed: 08-Oct-07
Page: 1
Acute Toxicity/Intravenous screen

Animal number	Sex	Group/ Subgroup	Date and time data was entered	Date data taken	Opr #	Tissue / Observation(s) Locator, Severity, Other, Distribution, Shape/Attachments, Texture
RT1F0482	F	1/1				GENERAL COMMENT
			21-Mar-07 14:52	21-Mar-07	57	All organs, no abnormality, , , , ,
RT1F0483	F	1/1				GENERAL COMMENT
			21-Mar-07 14:53	21-Mar-07	57	All organs, no abnormality, , , , ,
RT1F0484	F	1/1				GENERAL COMMENT
			21-Mar-07 14:53	21-Mar-07	57	All organs, no abnormality, , , , ,
RT1F0485	F	1/1				GENERAL COMMENT
			21-Mar-07 14:53	21-Mar-07	57	All organs, no abnormality, , , , ,
RT1F0486	F	1/1				GENERAL COMMENT
			21-Mar-07 14:54	21-Mar-07	57	All organs, no abnormality, , , , ,
RT2F0487	F	2/1				GENERAL COMMENT
			21-Mar-07 15:04	21-Mar-07	57	All organs, no abnormality, , , , ,
RT2F0488	F	2/1				GENERAL COMMENT
			21-Mar-07 15:04	21-Mar-07	57	All organs, no abnormality, , , , ,
RT2F0489	F	2/1				GENERAL COMMENT
			21-Mar-07 15:04	21-Mar-07	57	All organs, no abnormality, , , , ,
RT2F0490	F	2/1				GENERAL COMMENT
			21-Mar-07 15:04	21-Mar-07	57	All organs, no abnormality, , , , ,
RT2F0491	F	2/1				GENERAL COMMENT
			21-Mar-07 15:05	21-Mar-07	57	All organs, no abnormality, , , , ,
RT3F0492	F	3/1				GENERAL COMMENT
			21-Mar-07 15:16	21-Mar-07	57	All organs, no abnormality, , , , ,
RT3F0493	F	3/1				GENERAL COMMENT
			21-Mar-07 15:16	21-Mar-07	57	All organs, no abnormality, , , , ,
RT3F0494	F	3/1				GENERAL COMMENT
			21-Mar-07 15:16	21-Mar-07	57	All organs, no abnormality, , , , ,
RT3F0495	F	3/1				GENERAL COMMENT
			21-Mar-07 15:16	21-Mar-07	57	All organs, no abnormality, , , , ,

Animal number	Sex	Group/ Subgroup	Date and time data was entered	Date data taken	Opr #	Tissue / Observation(s) Locator, Severity, Other, Distribution, Shape/Attachments, Texture
RT3F0496	F	3/1				GENERAL COMMENT
			21-Mar-07 15:17	21-Mar-07	57	All organs, no abnormality, , , , ,
RT4F0497	F	4/1				GENERAL COMMENT
			21-Mar-07 15:20	21-Mar-07	57	All organs, no abnormality, , , , ,
RT4F0498	F	4/1				GENERAL COMMENT
			21-Mar-07 15:21	21-Mar-07	57	All organs, no abnormality, , , , ,
RT4F0500	F	4/1				GENERAL COMMENT
			21-Mar-07 15:21	21-Mar-07	57	All organs, no abnormality, , , , ,
RT4F0501	F	4/1				GENERAL COMMENT
			21-Mar-07 15:22	21-Mar-07	57	All organs, no abnormality, , , , ,
RT5F0502	F	5/1				GENERAL COMMENT
			07-Mar-07 15:24	07-Mar-07	57	All organs, no abnormality, , , , ,
RT5F0503	F	5/1				GENERAL COMMENT
			07-Mar-07 15:24	07-Mar-07	57	All organs, no abnormality, , , , ,
RT5F0504	F	5/1				GENERAL COMMENT
			07-Mar-07 15:25	07-Mar-07	57	All organs, no abnormality, , , , ,
RT5F0505	F	5/1				GENERAL COMMENT
			07-Mar-07 15:26	07-Mar-07	57	All organs, no abnormality, , , , ,
RT5F0506	F	5/1				GENERAL COMMENT
			07-Mar-07 15:25	07-Mar-07	57	All organs, no abnormality, , , , ,
RT6F0507	F	6/1				GENERAL COMMENT
			21-Mar-07 15:30	21-Mar-07	57	All organs, no abnormality, , , , ,
RT6F0508	F	6/1				GENERAL COMMENT
			21-Mar-07 15:31	21-Mar-07	57	All organs, no abnormality, , , , ,
RT6F0509	F	6/1				GENERAL COMMENT
			21-Mar-07 15:31	21-Mar-07	57	All organs, no abnormality, , , , ,
RT6F0510	F	6/1				GENERAL COMMENT
			21-Mar-07 15:31	21-Mar-07	57	All organs, no abnormality, , , , ,

Animal number	Sex	Group/ Subgroup	Date and time data was entered	Date data taken	Opr #	Tissue / Observation(s) Locator, Severity, Other, Distribution, Shape/Attachments, Texture
RT6F0511	F	6/1				GENERAL COMMENT
			21-Mar-07 15:31	21-Mar-07	57	All organs, no abnormality, , , , ,
RT7F0512	F	7/1				GENERAL COMMENT
			21-Mar-07 15:39	21-Mar-07	57	All organs, no abnormality, , , , ,
RT7F0513	F	7/1				GENERAL COMMENT
			21-Mar-07 15:39	21-Mar-07	57	All organs, no abnormality, , , , ,
RT7F0514	F	7/1				GENERAL COMMENT
			21-Mar-07 15:39	21-Mar-07	57	All organs, no abnormality, , , , ,
RT7F0515	F	7/1				GENERAL COMMENT
			21-Mar-07 15:40	21-Mar-07	57	All organs, no abnormality, , , , ,
RT7F0516	F	7/1				GENERAL COMMENT
			21-Mar-07 15:40	21-Mar-07	57	All organs, no abnormality, , , , ,

ATTACHMENTS

ATTACHMENT 1 - **PROTOCOL**

2mEPSPS protein
ACUTE TOXICITY BY INTRAVENOUS INJECTION IN MICE

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

SPONSOR:
Bayer AG
Bayer CropScience
Alfred Nobel Str. 50
40789 Monheim
Germany

1 GENERAL

1.1 PURPOSE OF STUDY

The objective of this study is to investigate the acute toxicity of the double mutated maize 5-enol pyruvylshikimate-3-phosphate synthase (2mEPSPS) protein after intravenous injection in mice.

1.2 GOOD LABORATORY PRACTICE COMPLIANCE

This study will not be subjected to specific Quality Assurance inspections. However, standardized, routine operating methods similar to those used on this type of study are periodically inspected.

1.3 REGULATORY GUIDELINES

This study is based on the U.S. E.P.A. Health Effects Test Guidelines OPPTS 870.1100, adopted in 2002 (1) and on the O.E.C.D. Test Guideline 425, adopted in 2001 (2).

2 STUDY PERSONNEL

2.1 STUDY DIRECTOR:

Date: 26 February 2007

D. ROUQUIE

2.2 SPONSOR REPRESENTATIVE:

Date: 26 FEBRUARY 2007

C. HERQUET-GUICHENEY

2.3 OTHER STUDY PERSONNEL

Responsibility		Name
In-life Supervisor	:	D. SANTAMARIA
Responsible Technician	:	S. LEBAS

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

3 PROPOSED DATES

Arrival of animals	:	February 28, 2007	
Experimental starting date	:	February 28, 2007	
Randomization	:	March 06, 2007	
Start of treatment	:	March 07, 2007	
Final sacrifice date	:	March 21, 2007	
Experimental completion date	:	March 30, 2007	(estimated)

4 OVERVIEW OF STUDY DESIGN

Groups of 5 female mice will be treated at two dose levels (1 and 10 mg/kg/body weight) in order to identify any acute toxic effects of the test protein.

Animals will be observed for clinical signs daily for 15 days. Body weight will be recorded at weekly intervals and on the day of necropsy. All mice, including those found dead or killed for humane reasons during the study, will be subjected to macroscopic observations and, when deemed appropriate by the Study Director or the Sponsor Representative, tissues will be retained for possible microscopic examinations.

5 TEST SYSTEM

5.1 SPECIES SPECIFICATIONS

5.1.1 Species and strain

Species: Mouse

Sex: Female

Strain: Crl:OF1

Body weight at study start: 20-30 g

5.1.2 Animal supplier

Charles River France Laboratories (Saint Germain sur l'Arbresle, France).

5.1.3 Reason for selection of species

The mouse has been chosen because of its acceptance by Regulatory Authorities as a test species to assess acute toxicity.

The OF1 strain has been used extensively in toxicity evaluation studies, hence sufficient background data exist to support interpretation of results.

5.1.4 Age range and number

Forty female mice will be ordered. Animals will be 7 weeks of age at the start of exposure to the test substance.

5.1.5 Acclimatization phase and randomization

The duration of the acclimatization phase will be at least 6 days.

Animals will be checked twice daily for moribundity and mortality except on weekends and public holidays when they will be checked once daily.

All animals will be weighed at least weekly during the acclimatization phase.

The acceptable body weight range will be ±20% of the mean body weight on the day of randomization.

Any animal deemed unsuitable for the study based on weight or clinical signs will not be used in the study.

From the remaining animals, five mice will be allocated to the dosage group by using a computerized randomization procedure that ensures a similar body weight distribution within this group.

5.1.6 Identification

At the time of randomization each animal will be identified by a stainless steel ear tag bearing a unique animal number.

5.2 DIET INFORMATION

5.2.1 Food

Certified rodent pelleted and irradiated diet A04C-10 from S.A.F.E. (Scientific Animals Food and Engineering, Augy, France) will be available *ad libitum*.

Food will be stored in an identified room controlled for temperature and humidity. Diet will be used only until date of expiry.

5.2.2 Water

Filtered and softened tap water from the municipal water supply will be provided *ad libitum* using automatic watering system. Filters servicing the watering system are changed regularly and sterilization of the system is periodically performed.

5.2.3 Analyses

Analytical data will be provided by the manufacturer for each batch of diet including the size of pellets and concentration of nutritional components, selected heavy metals, pesticides, mycotoxins, microorganisms and nitroso compounds. Batches of diet will be only released for use after confirmation they meet specification.

Certificates of water analysis will be provided by the "Laboratoire Municipal d'Hygiène de la Ville de Nice" (France) and "Institut d'Hygiène Alimentaire de Longjumeau" (France).

5.2.4 Records

Records of certificates of food and water analyses will be retained in the archives.

5.3 ENVIRONMENTAL CONDITIONS

5.3.1 Room

Animal room number: L8

The animal room is within a barrier maintained unit with restricted entry.

5.3.2 Housing

Animals will be housed individually in suspended, stainless steel, wire mesh cages. The cage of each animal will be identified by a card bearing a unique identification number.

5.3.3 General environment

Temperature, humidity and ventilation:
Laboratory conditions will be controlled to ensure a temperature of 20°C - 24°C and a relative humidity of 40% - 70% with a target of 10 to 15 air changes per hour.

Lighting:
12-hour light/dark cycles will be provided by automatically controlled fluorescent-tube lighting (7am - 7pm).

Monitoring:
The temperature, humidity and lighting in the animal room are constantly monitored by an automatic system. The ventilation system in the animal room is maintained to ensure adequate ventilation, with the performance of the system regularly checked. Records of all deviations from specifications will be placed in the study file.

6 CONTROL AND TEST SUBSTANCES

6.1 CONTROL SUBSTANCES

Negative and positive control with known toxicity diluted in PBS buffer (13.7 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.2 mM KH2PO4, pH 7.4) will be used as reference compounds.

Aprotinin will be used as a negative control (protein with molecular weight 6500 daltons, reference A4529 Sigma-Aldrich, Saint Quentin Fallavier, France). Aprotinin is a serin protease inhibitor, non toxic when administered by intravenous route up to 10 mg/kg in mice.

Melittin will be used as positive control (protein with molecular weight 2840 daltons, reference M2272 Sigma-Aldrich, Saint Quentin Fallavier, France). Melittin is the principle hemolytic component of honey bee, highly toxic when administered by intravenous route up to 3 mg/kg or higher in mice.

A control group will be administred the vehicle alone PBS buffer.

6.2 TEST SUBSTANCE CHARACTERISTICS

6.2.1 Identification of test substance

Test substance	:	2mEPSPS protein
Batch number	:	LEJ5838
Purity	:	> 95 %
Concentration	:	1.08 mg/ml in PBS buffer
Certified through	:	At least 6 months at -20°C

Full details of the test substance description including its chemical structure and physical appearance will be included in the final report.

Test substance name: Double mutated maize 5-enol pyruvylshikimate-3-phosphate synthase (2mEPSPS) protein encoded by the *2mepsps* gene produced in *Escherichia Coli*.

The test substance will be supplied by the Sponsor (Bayer BioScience NV, Gent, Belgium). The storage of a retention sample is under the responsibility of the Sponsor.

6.2.2 Activity

The 2mEPSPS protein confers to the modified plant herbicide (Glyphosate) tolerance properties. Glyphosate's herbicidal activity is conferred by its ability to potently inhibit the wild-type plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which has an essential function in all plants, fungi, and bacteria in the biosynthesis of aromatic amino acids. The *2mepsps* gene was generated by introducing mutations into the wild-type *epsps* (wt *epsps*) gene from maize. Alteration of the gene leads to a double mutant EPSPS protein containing two amino acid substitutions (2mEPSPS). These modifications confer on the protein a decreased binding affinity for glyphosate, allowing it to maintain sufficient enzymatic activity in the presence of the herbicide. Therefore, the plants bearing this gene become tolerant to glyphosate herbicide.

6.2.3 Storage

The test substance will be stored frozen in an air-tight, light-resistant container at approximately -20°C or according to the conditions described in the test substance specifications when available. The storage stability is certified through at least 6 months at -20°C for the control and test substances.

6.2.4 Safety handling and requirements

Information on the appropriate safety precautions when handling the test substance will be given by the supplier or the Sponsor Representative.

In the absence of information on the potential toxic effects of the control and test substances, safety precautions will be applied according to the relevant standard operating procedures.

6.2.5 Analyses

The confirmation of the identity, purity and activity of the controls and the test substances will be provided by the supplier and the Sponsor Representative respectively. The certificate of analysis of the test substance will be placed in the final study report.

6.3 TEST SUBSTANCE FORMULATION

6.3.1 Preparation, shipment and storage conditions

The test substance will be received from the Sponsor as a formulated solution. This formulated solution is under the responsibility of the Sponsor.

The formulation will be shipped under frozen conditions and will be stored at -20°C at arrival. On the day of dosing, it will be thawed and homogenized before administration. The unused residue of the formulation will be stored at -20°C at the end of the administration period.

6.3.2 Analyses

Stability and homogeneity of the formulated test substance will be confirmed analytically. If the test substance is soluble in the used formulation, homogeneity will not be confirmed analytically. Stability and homogeneity of the formulated test substance will not be confirmed analytically if test substance is known to be stable and homogenous in both undiluted and in-ready-to-use dilution with distilled water (e.g., commercial product).

7 ROUTE OF ADMINISTRATION AND TREATMENT GROUPS

7.1 CHOICE OF DOSES

The doses of 1 and 10 mg 2mEPSPS protein/ kg body weight were selected after discussion with the Sponsor Representative.

This choice was based on the preliminary safety assessment of the test substance which led to the conclusion with a high degree of certainty of the lack of harmful effects caused by the 2mEPSPS protein after intravenous administration to mammals.

7.2 CHOICE OF ROUTE OF ADMINISTRATION

The intravenous route was selected to ensure exposure to the test substance and was based on previous information obtained in acute mouse studies. Historically, the intravenous route of exposure has been used to investigate the toxicity of many proteins.

7.3 NUMBER OF ANIMALS

Five female mice per group will be administered with the appropriate concentrations of control or the test substances.

7.4 CONDITIONS OF ADMINISTRATION

All groups used in the study will receive the appropriate concentrations of control or test substance in vehicle at a constant volume of 10 ml/kg.

7.5 DOSES

GROUP	CONTROL OR TEST SUBSTANCE(S)	DOSE LEVELS mg/kg	NUMBER OF FEMALE ANIMALS PER GROUP	ANIMALS IDENTITY FEMALES
1	Negative control: vehicle	0	5	RT1F0482 to 0486
2	Negative control: Aprotinin	1	5	RT2F0487 to 0491
3		10	5	RT3F0492 to 0496
4	Positive control: Melittin	1	5	RT4F0497 to 0501
5		10	5	RT5F0502 to 0506
6	Test substance: 2mEPSPS protein	1	5	RT6F0507 to 0511
7		10	5	RT7F0512 to 0516

7.6 ANIMAL WELFARE

During the study the care and use of animals will be conducted in accordance with the regulations of the Guide for the Care and Use of Laboratory Animals (Public Health Service, National Institute of Health, NIH publication N°86-23, revised 1985) and "Le Guide du Journal Officiel des Communautés Européennes L358, 18 Décembre 1986, n°86/609/CEE du 24 Novembre 1986".

8 LABORATORY DETERMINATIONS AND SCHEDULES

All animal data will be recorded using a dedicated computer system (Path/Tox System version 4.2.2, protocol number RP-0715).

8.1 CLINICAL EXAMINATION

8.1.1 Clinical signs and mortality

Clinical signs will be recorded individually starting on Day -1, at least once during the first 30 minutes after each dosing, periodically during the first 24 hours post-dosing, and every day thereafter through Day 15. Additional observations will be necessary if the animals continue to display signs of toxicity. The nature, onset, severity, reversibility and duration of clinical signs will be recorded.

During the acclimatization phase and throughout the study, animals will be checked twice daily for moribundity and mortality (once daily except on weekends and public holidays). Any animal suffering from severe distress, in a moribund condition or considered unlikely to survive will be humanely killed, and will be considered in the interpretation of the test results in the same way as animals that died on test.

8.1.2 Body weight

Body weights will be measured on study Days -5, -1, 1 (shortly before the test substance is administered), 8 and 15. Additionally, animals will be weighed when killed for humane reasons or when found dead.

8.2 POST MORTEM EXAMINATION

8.2.1 Necropsy procedures

Animals found dead:

Any animal found dead during the study will be necropsied as soon as possible but within 24 hours of the time of discovery. If necropsy cannot be performed immediately after a dead animal is discovered, the animal should be refrigerated (not frozen).

Scheduled sacrifice and moribund animals:

Animals surviving to the end of the study and animals sent to necropsy for humane reasons will be deeply anesthetized at the end of the study by Isoflurane (T.E.M., Lormont, France) inhalation, then exsanguinated before necropsy.

8.2.2 Necropsy

The necropsy of animals will include the macroscopic examination of the external surface, all orifices and all major body cavities and organs.

Significant macroscopic findings will be recorded. Tissues may be sampled at the discretion of the Study Director or the Sponsor Representative.

8.2.3 Microscopic evaluation

Microscopic examination of organs showing evidence of gross pathology in animals surviving at least 24 hours may be analyzed.

9 CALCULATIONS

For body weights, means and standard deviations will be calculated when survival exceeds 1 day.

10 REPORTING

10.1 INTERIM REPORTS

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

10.2 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 into the final report. The final report will include the information and data required by the referenced guidance documents (1, 2).

11 ARCHIVING

All raw data, supporting documents, as well as protocol, protocol amendments, protocol deviations and the final report will be maintained in the archive room. An aliquot of the test substance reference samples and will be maintained in the archived sample room.

All of the above will be saved for at least ten years in the designated areas at:

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

12 REFERENCES

12.1 GENERAL REFERENCES

1 - U.S. E.P.A. (United States Environmental Protection Agency), 1998. Prevention, Pesticides and Toxic Substances (7101), Health Effects Test Guidelines OPPTS 870.1100, Acute Oral Toxicology, EPA 712-C-98-190, December 2002, 35 pages.

2 - O.E.C.D. (Organization for Economic Co-operation and Development), 2001. O.E.C.D. Guideline for Testing of Chemicals, Test Guideline N°425: Acute Oral Toxicity – Up-and-Down Procedure. December 17, 2001, 26 pages.

ATTACHMENT 2 - **CERTIFICATES OF ANALYSIS**

Bayer HealthCare

Certificate of Analysis

5-Enolpyruvylshikimate-3-phosphate synthase (2mEPSPS)
(Lot-no. LEJ5838)

Bayer HealthCare AG, Product Supply, Biotechnologie
Friedrich-Ebert-Straße 217, D-42096 Wuppertal

Protein: 2mEPSPS

Sequence (theor.):

AGAEEIVLQPIKEISGTVKLPGSKSLSNRILLLAALSEGTTVVDNLLNSEDVH
YMLGALRTLGLSVEADKAAKRAVVVGCGGKFPVEDAKEEVQLFLGNAGIAMRS
LTAAVTAAGGNATYVLDGVPRMRERPIGDLVVGLKQLGADVDCFLGTDCPPVR
VNGIGGLPGGKVKLSGSISSQYLSALLMAAPLALGDVEIEIIDKLISIPYVEM
TLRLMERFGVKAEHSDSWDRFYIKGGQKYKSPKNAYVEGDASSASYFLAGAAI
TGGTVTVEGCGTTSLQGDVKFAEVLEMMGAKVTWTETSVTVTGPPREPFGRKH
LKAIDVNMNKMPDVAMTLAVVALFADGPTAIRDVASWRVKETERMVAIRTELT
KLGASVEEGPDYCIITPPEKLNVTAIDTYDDHRMAMAFSLAACAEVPVTIRDP
GCTRKTFPDYFDVLSTFVKN

Molecular weight: 47415.75 Da

Lot-no.: LEJ5838

Delivered amount: **108 mg**
- frozen solution; storage temperature: -80 to -70 °C
- 1 bottle with 108 mg

0.5 mg
- 1 bottle with 0.5 mg/bottle (incoming testing sample)

Concentration: 1.08 mg/mL
(OD measurement at λ = 280 nm; extinction coefficient: 33265 M^{-1} cm^{-1})

SDS-PAGE Analysis:
- protocol and accomplishment laboratory Dr. W. Schröder
- under reducing conditions, Coomassie blue staining
- see fig. 1 (page 2)

Western Blot Analysis:
- protocol and accomplishment laboratory Dr. M. Dörschug
- under reducing conditions
- see fig. 2 (page 2)

Sequencing:
- protocol and accomplishment laboratory Dr. W. Schröder
- see page 3

Bayer HealthCare

Certificate of Analysis (Continuation)

5-Enolpyruvylshikimate-3-phosphate synthase (2mEPSPS)
(Lot-no. LEJ5838)

Bayer HealthCare AG, Product Supply, Biotechnologie
Friedrich-Ebert-Straße 217, D-42096 Wuppertal

SDS-PAGE Analysis:

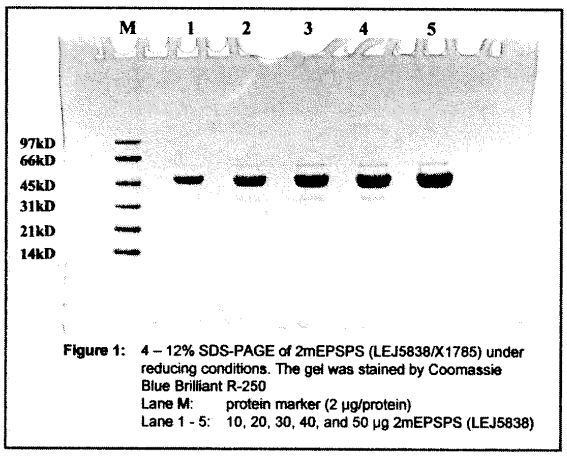

Figure 1: 4 – 12% SDS-PAGE of 2mEPSPS (LEJ5838/X1785) under reducing conditions. The gel was stained by Coomassie Blue Brilliant R-250
Lane M: protein marker (2 µg/protein)
Lane 1 - 5: 10, 20, 30, 40, and 50 µg 2mEPSPS (LEJ5838)

Western Blot Analysis:

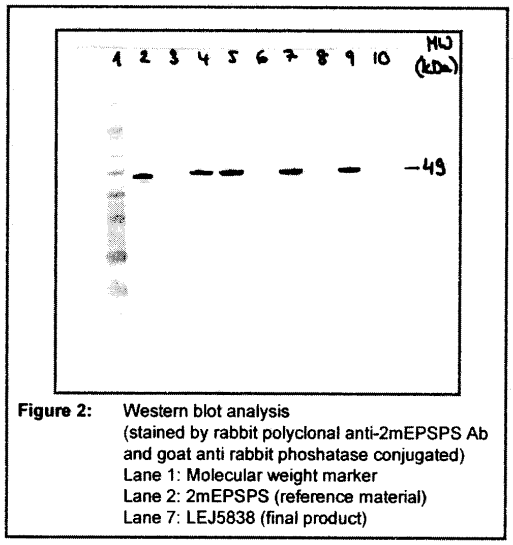

Figure 2: Western blot analysis
(stained by rabbit polyclonal anti-2mEPSPS Ab and goat anti rabbit phoshatase conjugated)
Lane 1: Molecular weight marker
Lane 2: 2mEPSPS (reference material)
Lane 7: LEJ5838 (final product)

Bayer HealthCare

Certificate of Analysis (Continuation)

5-Enolpyruvylshikimate-3-phosphate synthase (2mEPSPS)
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Sequencing: Purity N-terminal sequence: > 90%

Sum of two sequences:

1. AGAEEIVLQPIKEI.....	74%
2. MAGAEEIVLQPIKE.....	17%

Wuppertal, 2006-06-01

Dr. Jürgen Lenz

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Aprotinin from bovine lung, lyophilized powder, 3-7 TIU/mg solid
Product Number	A4529
Product Brand	Sigma-Aldrich
CAS Number	9087-70-1
Molecular Formula	$C_{284}H_{432}N_{84}O_{79}S_7$
Molecular Weight	6511.44
Storage Temp	2-8°C

TEST	SPECIFICATION	LOT 065K7013 RESULTS
APPEARANCE	REPORT RESULT	WHITE LYOPHILIZED POWDER
ACTIVITY	3 TO 7 TIU/MG SOLID	4.3TIU/MG SOLID
UNIT DEFINITION	ONE TRYPSIN INHIBITOR UNIT (TIU) WILL DECREASE THE ACTIVITY OF 2TRYPSIN UNITS BY 50% WHERE ONE TRYPSIN UNIT WILL HYDROLYZE 1.0MICROMOLE OF N-ALPHA-BENZOYL-DL-ARGININE P-NITROANILIDE (BAPNA) PER MINUTE AT PH7.8 AT 25DEGC.	
RECOMMENDED RETEST	2 YEARS	JUNE 2008 EXTENDED FROM JUNE 2007
QC RELEASE DATE		JUNE 2007

Rodney Burbach, Supervisor
Analytical Services
St. Louis, Missouri USA

SIGMA-ALDRICH

Certificate of Analysis

Product Name	Melittin from honey bee venom, ≥85% (HPLC)
Product Number	M2272
Product Brand	Sigma
CAS Number	37231-28-0
Molecular Formula	$C_{131}H_{229}N_{39}O_{31}$
Molecular Weight	2846.46
Storage Temp	−20°C

TEST	SPECIFICATION	LOT 125K4104 RESULTS
APPEARANCE	WHITE TO FAINT YELLOW WITH A FAINT TAN CAST POWDER	FAINT TAN POWDER
SOLUBILITY	CLEAR COLORLESS TO FAINT YELLOW SOLUTION AT 5MG/ML IN PBS OR WATER	CLEAR FAINT YELLOW
PHOSPHOLIPASE A2 CONTENT	< OR = 0.5 UNITS/MG SOLID	1 UNIT/MG SOLID
PURITY BY HPLC	> OR = 85%	93%
QC RELEASE DATE		DECEMBER 2005

Rodney Burbach, Supervisor
Analytical Services
St. Louis, Missouri USA

FINAL REPORT AMENDMENT

There is no final report amendment at this time.

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