C 021395

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DOCMAP N°605512

PAT (PHOSPHOACETYL TRANSFERASE) PROTEIN DERIVED FROM *bar* GENE

ACUTE TOXICITY BY INTRAVENOUS INJECTION IN THE MOUSE

REPORT OF STUDY SA 01352

STUDY DIRECTOR: P. KENNEL

PERFORMING LABORATORY:

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355, rue Dostoïevski
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STUDY COMPLETED ON: MARCH 13; 2002

FINAL REPORT AMENDMENT

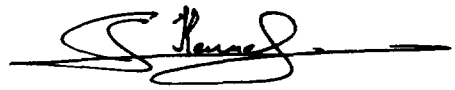
There is no final report amendment at this time.

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 test material production and that the dosing suspensions were not analyzed for concentration, homogeneity or stability.

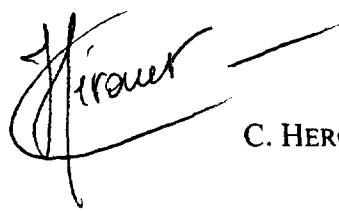
- O.E.C.D. (Organization for Economic Cooperation and Development) Principles of Good Laboratory Practice, 1997.
- European Commission Directive 1999/11/EC, 1999.
- French decree n° 98-1312, regarding Good Laboratory Practice, December 31, 1998.
- E.P.A. (Environmental Protection Agency)
  - 40 CFR part 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.), notification 12 NohSan n°8628, (December 06, 2000).

Study Director:



Date: March 13, 2002 P. KENNEL

Sponsor Representative:



Date: March 13, 2002 C. HEROUET

Study Submitter:

Date:

QUALITY ASSURANCE STATEMENT

The conduct of the study has been subjected to periodic inspections by the Aventis 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	December 26, 2001	January 07, 2002	January 07, 2002
Animal weighing Treatment administration	January 10, 2002	January 10, 2002	January 11, 2002
Report	February 28, 2002	February 28, 2002	March 12, 2002

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: March 13, 2002

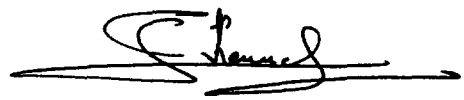
G. ODAGLIA

SIGNATURES

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.

Study Director:



Date: March 13, 2002

P. KENNEL

STUDY PROFESSIONALS

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

STUDY DIRECTOR	:	P. KENNEL
LABORATORY ANIMAL RESOURCES	:	J.P. KOCWIN
TOXICOLOGY SUPERVISOR	:	B. BONNAFOUS
RESPONSIBLE TECHNICIAN	:	S. COLOMBEL
REPORT UNIT ASSISTANT	:	M. VAGNER

TABLE OF CONTENTS

SUMMARY

The objective of this study was to assess the acute intravenous toxicity in OF1 mice of PAT (phosphoacetyl transferase) protein (batch number 220601701, >95% purity), a protein encoded by the *bar* gene. In addition, the acute intravenous toxicity of aprotinin (negative control) and melittin (positive control) were also compared. Groups of 5 female OF1 mice were administered either PAT protein, aprotinin or melittin in physiological saline at dose levels of 1 and 10 mg/kg body weight. 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.

There were no mortality or treatment-related toxic effects in female OF1 mice after acute intravenous administration of PAT (phosphoacetyl transferase) protein at 1 and 10 mg/kg.

By contrast, positive control (melittin), at 10 mg/kg, induced 100% mortality. Animals treated at 1 mg/kg of melittin and negative control animals treated with aprotinin at 1 and 10 mg/kg showed no visible signs of systemic toxicity.

INTRODUCTION

The objective of this study were to assess the potential for acute intravenous toxicity of PAT protein derived from *bar* gene in the OF1 mouse. In addition, the acute intravenous toxicity of aprotinin (negative control) and melittin (positive control) were also compared.

The study time schedule was as follows:

Study initiation date *	December 19, 2001
Sponsor protocol approval date	December 19, 2001
Animal arrival date	January 03, 2002
Experimental starting date	January 03, 2002
Randomization date (Day -1)	January 09, 2002
Dosing dates	January 10, 2002
First final sacrifice date	January 24, 2002
Experimental completion date	January 24, 2002

* Date of protocol approval by Study Director.

MATERIAL AND METHODS

1 - CONTROL AND TEST SUBSTANCE FORMULATION

The test substance PAT (phosphoacetyl transferase) protein (batch number 220601701, >95% purity), a protein derived from *bar* gene was used in this study. Information on the chemical characterization of the test substance was documented by Aventis CropScience, Gent, Belgium and is presented in Att. 3. The test substance was stored in an air-tight, light resistant container at approximately minus 70°C.

The aprotinin protein, a serin protease inhibitor (molecular weight 6 511 daltons, reference number A4529, batch number 080K7022) was used as negative control. Chemical information on this negative control was documented by Sigma-Aldrich, Saint-Quentin Fallavier, France and is presented in Att. 3. This negative control was stored in an air-tight, light-resistant container at approximately minus 20°C.

The melittin protein, the principle hemolytic component of honey bee venom (molecular weight 2 847 daltons, reference number M2272, batch number 021K4008, 93% purity) was used as positive control. Chemical information on this positive control was documented by Sigma-Aldrich, Saint-Quentin Fallavier, France and is presented in Att. 3. The positive control was stored in an air-tight, light-resistant container at approximately minus 20°C.

The formulations were prepared by dissolving the substances in sterilized physiological saline to produce the required concentration (w/v or v/v). The formulations were placed in glass air-tight 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 its recommendation by regulatory authorities as an appropriate test species to assess acute intravenous toxicity. The OF1 strain was used since sufficient background toxicity data exist to support interpretation of results. A total of 42 female OF1 (IOPS Caw) mice were obtained from Iffa-Credo, l'Arbresle, France. Animals were acclimatized to laboratory conditions for 7 days prior to the treatment and were 7 weeks old at the start of treatment.

a/Selection and randomization

All animals were examined for mortality during the acclimatization phase. The day before the test substance administration, all suitable animals were weighed. Thirty-five female mice were selected for the study. An automatic procedure was used to select animals for the study from the middle of the weight range of the available animals. Selected animals were in a weight range from 24.3 to 29.5 g on the day of treatment. Body weights were within ±20% of the mean body weight on the day of randomization. Animals not used in the study were maintained as stock animals within the animal facility.

b/Identification

On the day before treatment, 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. Each cage was identified by a card specifying the study number, treatment group and dosage. The laboratory conditions in the study room were controlled and 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)
* ventilation: 15 air changes per hour (average, not monitored).

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 U.A.R. (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France) and filtered and softened water from the municipal water supply, were available *ad libitum*. 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 report of the physicochemical properties and concentration of specified contaminants are 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 materials were administered in sterile 0.9% sodium chloride (physiological saline ; vehicle), intravenously through the tail vein at a volume of 12 ml/kg (based on body weight on Day 1).

Details of group sizes and treatments:

GROUPS	CONTROL OR TEST SUBSTANCES	DOSE LEVELS mg/kg	ANIMAL IDENTITY	NUMBER OF FEMALE ANIMALS PER GROUP
1	Negative control: vehicle	0	LT1F6172 to 6176	5
2	Negative control: aprotinin	1	LT2F6177 to 6181	5
3		10	LT3F6182 to 6186	5
4	Positive control: melittin	1	LT4F6187 to 6191	5
5		10	LT5F6192 to 6196	5
6	Test substance: PAT protein	1	LT6F6197 to 6201	5
7		10	LT7F6202 to 6206	5

4 - DAILY OBSERVATIONS

Clinical signs were recorded daily from Day 1 through Day 15. They were recorded approximately 1 hour after 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 week-end and public holidays when they were checked once daily.

5 - BODY WEIGHT

Each animal was weighed on Days -6, -1, 1, 8 and 15 or when found dead.

6 - POST MORTEM PROCEDURES

All animals were autopsied. At final sacrifice on Day 15, surviving animals were anesthetized by intraperitoneal injection of pentobarbital, 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.

7 - CALCULATIONS

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

8 - 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:

Aventis CropScience
355, rue Dostoïevski
BP 153
F-06903 Sophia Antipolis Cedex

RESULTS

1 - MORTALITY (Tab. 1)

Group mortality:

Groups	Control or Test substances	Dose levels mg/kg	Number of dead exposed female animals
1	Negative control: vehicle	0	0/5
2	Negative control:	1	0/5
3	aprotinin	10	0/5
4	Positive control:	1	0/5
5	melittin	10	5/5
6	Test substance:	1	0/5
7	PAT protein	10	0/5

Mortality was observed on Day 1 for all positive control animals treated at 10 mg/kg of melittin. Clinical signs recorded in one of these animals before death consisted of prostration, slow respiration and hypothermia. No mortality was observed during the study in PAT protein-treated animals at 1 and 10 mg/kg or in other control groups.

2 - DAILY OBSERVATIONS (Tab. 1)

No clinical signs were noted in PAT protein-treated animals or in control groups throughout the study period.

3 - BODY WEIGHT (Tab. 2, 3)

The body weight evolution was unaffected by the treatment with either PAT protein at 1 and 10 mg/kg or control substances up to Day 15.

4 - GROSS PATHOLOGY (Tab. 4)

No treatment-related macroscopic abnormalities were detected in animals treated with either PAT protein at 1 and 10 mg/kg or control substances.

CONCLUSION

There were no mortality or treatment-related toxic effects in female OF1 mice after acute intravenous administration of PAT (phosphoacetyl transferase) protein at 1 and 10 mg/kg.

By contrast, positive control (melittin), at 10 mg/kg, induced 100% mortality. Animals treated at 1 mg/kg of melittin and negative control animals treated with aprotinin at 1 and 10 mg/kg showed no visible signs of systemic toxicity.

PROTOCOL DEVIATIONS

There were no protocol deviations during the study.

Study Director:

Date: March 13, 2002

P. KENNEL

ABBREVIATIONS

%	percentage
°C	degree Celsius
g	gram
ml/kg	milliliter/kilogram
mg/kg	milligram/kilogram
w/v	weight/volume
v/v	volume/volume
am	*ante meridiem*
pm	*post meridiem*
PAT	phosphoacetyl transferase
QA	Quality Assurance
GLP	Good Laboratory Practice
O.E.C.D.	Organization for Economic Cooperation and Development
E.P.A.	Environmental Protection Agency
M.A.F.F.	Ministry of Agriculture, Forestry and Fisheries
E.E.C.	European Economic Communities

TABLES

TABLE 1 - INDIVIDUAL CLINICAL SIGNS AND DEAD ANIMAL STATUS

Groups	Control or Test substances	Dose levels mg/kg
1	Negative control: vehicle	0
2	Negative control: aprotinin	1
3		10
4	Positive control: melittin	1
5		10
6	Test substance: PAT protein	1
7		10

Missing clinical signs data concerned dead animals.

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Center of Toxicology
Sophia-Antipolis
Mouse/OF 1

INDIVIDUAL CLINICAL SIGNS TABLE
Study number: SA 01352
DATES 10-Jan-02 TO 24-Jan-02
Study start date: 10-Jan-02

Printed: 24-Jan-02
Page: 1
Acute Toxicity/Intravenous screen

DOSAGE LEVEL IN: mg/kg

ANIMAL		OBSERVATION	DAYS OBSERVED
LT1F6172	0.00	NORMAL THROUGHOUT INTERVAL	
LT1F6173	0.00	NORMAL THROUGHOUT INTERVAL	
LT1F6174	0.00	NORMAL THROUGHOUT INTERVAL	
LT1F6175	0.00	NORMAL THROUGHOUT INTERVAL	
LT1F6176	0.00	NORMAL THROUGHOUT INTERVAL	
LT2F6177	1.00	NORMAL THROUGHOUT INTERVAL	
LT2F6178	1.00	NORMAL THROUGHOUT INTERVAL	
LT2F6179	1.00	NORMAL THROUGHOUT INTERVAL	
LT2F6180	1.00	NORMAL THROUGHOUT INTERVAL	
LT2F6181	1.00	NORMAL THROUGHOUT INTERVAL	
LT3F6182	10.00	NORMAL THROUGHOUT INTERVAL	
LT3F6183	10.00	NORMAL THROUGHOUT INTERVAL	
LT3F6184	10.00	NORMAL THROUGHOUT INTERVAL	
LT3F6185	10.00	NORMAL THROUGHOUT INTERVAL	
LT3F6186	10.00	NORMAL THROUGHOUT INTERVAL	
LT4F6187	1.00	NORMAL THROUGHOUT INTERVAL	
LT4F6188	1.00	NORMAL THROUGHOUT INTERVAL	
LT4F6189	1.00	NORMAL THROUGHOUT INTERVAL	
LT4F6190	1.00	NORMAL THROUGHOUT INTERVAL	
LT4F6191	1.00	NORMAL THROUGHOUT INTERVAL	
LT5F6196	10.00	Movement/Behavior, prostration	1
		Respiration, slow respiration	1
		General appearance, cold to touch	1
LT6F6197	1.00	NORMAL THROUGHOUT INTERVAL	

DOSAGE LEVEL IN: mg/kg

ANIMAL		OBSERVATION	DAYS OBSERVED
LT6F6198	1.00	NORMAL THROUGHOUT INTERVAL	
LT6F6199	1.00	NORMAL THROUGHOUT INTERVAL	
LT6F6200	1.00	NORMAL THROUGHOUT INTERVAL	
LT6F6201	1.00	NORMAL THROUGHOUT INTERVAL	
LT7F6202	10.00	NORMAL THROUGHOUT INTERVAL	
LT7F6203	10.00	NORMAL THROUGHOUT INTERVAL	
LT7F6204	10.00	NORMAL THROUGHOUT INTERVAL	
LT7F6205	10.00	NORMAL THROUGHOUT INTERVAL	
LT7F6206	10.00	NORMAL THROUGHOUT INTERVAL	

NOTE: DATA FOR Dosing phase

Aventis CropScience
Center of Toxicology
Sophia-Antipolis

Dead Animal Status List for All Animals
Study number: SA 01352

Printed: 24-Jan-02
Page: 1

Mouse/OF 1 — Study start date: 10-Jan-02 — 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	Status	Term. Body Wt. (g)	Ow	Grs
LT1F6172	1	F	Dosing phase	24-Jan-02 09:41	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT1F6173	1	F	Dosing phase	24-Jan-02 09:42	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT1F6174	1	F	Dosing phase	24-Jan-02 09:42	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT1F6175	1	F	Dosing phase	24-Jan-02 09:42	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT1F6176	1	F	Dosing phase	24-Jan-02 09:43	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT2F6177	2	F	Dosing phase	24-Jan-02 09:43	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT2F6178	2	F	Dosing phase	24-Jan-02 09:43	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT2F6179	2	F	Dosing phase	24-Jan-02 09:44	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT2F6180	2	F	Dosing phase	24-Jan-02 09:44	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT2F6181	2	F	Dosing phase	24-Jan-02 09:45	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT3F6182	3	F	Dosing phase	24-Jan-02 09:45	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT3F6183	3	F	Dosing phase	24-Jan-02 09:46	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT3F6184	3	F	Dosing phase	24-Jan-02 09:46	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT3F6185	3	F	Dosing phase	24-Jan-02 09:46	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT3F6186	3	F	Dosing phase	24-Jan-02 09:47	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT4F6187	4	F	Dosing phase	24-Jan-02 09:47	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT4F6188	4	F	Dosing phase	24-Jan-02 09:47	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT4F6189	4	F	Dosing phase	24-Jan-02 09:48	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT4F6190	4	F	Dosing phase	24-Jan-02 09:48	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT4F6191	4	F	Dosing phase	24-Jan-02 09:49	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT5F6192	5	F	Dosing phase	10-Jan-02 11:10	98	10-Jan-02	1	u	Found dead	27.2	-	C
LT5F6193	5	F	Dosing phase	10-Jan-02 11:11	98	10-Jan-02	1	u	Found dead	26.0	-	C
LT5F6194	5	F	Dosing phase	10-Jan-02 11:11	98	10-Jan-02	1	u	Found dead	27.7	-	C
LT5F6195	5	F	Dosing phase	10-Jan-02 11:12	98	10-Jan-02	1	u	Found dead	25.6	-	C
LT5F6196	5	F	Dosing phase	10-Jan-02 11:26	98	10-Jan-02	1	u	Found dead	24.2	-	C
LT6F6197	6	F	Dosing phase	24-Jan-02 09:50	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT6F6198	6	F	Dosing phase	24-Jan-02 09:50	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT6F6199	6	F	Dosing phase	24-Jan-02 09:51	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT6F6200	6	F	Dosing phase	24-Jan-02 09:51	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT6F6201	6	F	Dosing phase	24-Jan-02 09:51	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT7F6202	7	F	Dosing phase	24-Jan-02 09:52	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT7F6203	7	F	Dosing phase	24-Jan-02 09:52	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT7F6204	7	F	Dosing phase	24-Jan-02 09:52	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT7F6205	7	F	Dosing phase	24-Jan-02 09:53	98	24-Jan-02	15	s	Final phase sacrifice	-----	-	C
LT7F6206	7	F	Dosing phase	24-Jan-02 09:53	98	24-Jan-02	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

Groups	Control or Test substances	Dose levels mg/kg
1	Negative control: vehicle	0
2	Negative control: aprotinin	1
3		10
4	Positive control: melittin	1
5		10
6	Test substance: PAT protein	1
7		10

Non recorded values concerned dead animals.

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Center of Toxicology
Sophia-Antipolis

ANIMAL BODY WEIGHTS IN (G)
Study number: SA 01352

Printed: 04-Feb-02
Page: 1

Mouse/OF 1 — Study start date: 10-Jan-02 — Acute Toxicity/Intravenous screen

DOSAGE IN mg/kg / ANIMAL		SEX	DAY OF PHASE 1	DAY OF PHASE 8	DAY OF PHASE 15
			FEMALE ANIMALS		
LT1F6172	0.00	F	24.7	25.6	28.3
LT1F6173			26.9	27.0	27.2
LT1F6174			26.6	27.6	27.3
LT1F6175			28.6	29.7	28.7
LT1F6176			25.2	26.4	27.3
		(n)	5	5	5
		MEANS	26.4	27.3	27.8
		SDEVS	1.5	1.6	0.7
LT2F6177	1.00	F	26.9	26.2	30.8
LT2F6178			27.1	27.9	29.9
LT2F6179			27.8	29.5	29.4
LT2F6180			24.7	26.2	27.3
LT2F6181			25.3	28.7	28.1
		(n)	5	5	5
		MEANS	26.4	27.7	29.1
		SDEVS	1.3	1.5	1.4
LT3F6182	10.00	F	24.8	28.2	27.9
LT3F6183			28.7	29.7	30.9
LT3F6184			25.7	26.6	28.5
LT3F6185			27.0	26.4	27.3
LT3F6186			25.7	27.3	26.6
		(n)	5	5	5
		MEANS	26.4	27.6	28.2
		SDEVS	1.5	1.4	1.6
LT4F6187	1.00	F	26.0	25.1	27.4
LT4F6188			26.2	26.7	29.0
LT4F6189			29.5	29.0	31.1
LT4F6190			27.0	29.0	30.8
LT4F6191			24.6	25.8	26.4
		(n)	5	5	5
		MEANS	26.7	27.1	28.9
		SDEVS	1.8	1.8	2.1
LT5F6192	10.00	F	27.2		
LT5F6193			26.0		
LT5F6194			27.5		
LT5F6195			25.5		

NOTE: DATA FOR Dosing phase

DOSAGE IN mg/kg ANIMAL		SEX	DAY OF PHASE 1	8	15
			FEMALE ANIMALS		
LT5F6196	10.00	F	24.3		
		(n)	5		
		MEANS	26.1		
		SDEVS	1.3		
LT6F6197	1.00	F	26.3	26.6	29.5
LT6F6198			26.2	27.2	29.4
LT6F6199			24.8	25.6	27.8
LT6F6200			28.3	29.6	28.4
LT6F6201			24.9	27.2	26.6
		(n)	5	5	5
		MEANS	26.1	27.2	28.3
		SDEVS	1.4	1.5	1.2
LT7F6202	10.00	F	27.0	27.9	29.6
LT7F6203			26.8	28.7	28.1
LT7F6204			26.4	26.9	28.5
LT7F6205			24.8	26.3	28.1
LT7F6206			25.3	27.9	29.7
		(n)	5	5	5
		MEANS	26.1	27.5	28.8
		SDEVS	1.0	0.9	0.8

NOTE: DATA FOR Dosing phase

TABLE 3 - MEAN AND INDIVIDUAL ABSOLUTE WEIGHT GAINS

Groups	Control or Test substances	Dose levels mg/kg
1	Negative control: vehicle	0
2	Negative control: aprotinin	1
3		10
4	Positive control: melittin	1
5		10
6	Test substance: PAT protein	1
7		10

Non recorded values concerned dead animals.

Aventis CropScience
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Sophia-Antipolis
House/OF 1

ANIMAL ABSOLUTE WEIGHT GAINS IN (G)
Study number: SA 01352
ABSOLUTE WEIGHT GAINS REFERENCED TO Dosing phase (DAY 1)
Study start date: 10-Jan-02

Printed: 04-Feb-02
Page: 1
Acute Toxicity/Intravenous screen

DOSAGE IN mg/kg ANIMAL		SEX	DAY OF PHASE 8	15
			FEMALE ANIMALS	
LT1F6172	0.00	F	0.9	3.6
LT1F6173			0.1	0.3
LT1F6174			1.0	0.7
LT1F6175			1.1	0.1
LT1F6176			1.2	2.1
		(n)	5	5
		MEANS	0.9	1.4
		SDEVS	0.4	1.5
LT2F6177	1.00	F	-0.7	3.9
LT2F6178			0.8	2.8
LT2F6179			1.7	1.6
LT2F6180			1.5	2.6
LT2F6181			3.4	2.8
		(n)	5	5
		MEANS	1.3	2.7
		SDEVS	1.5	0.8
LT3F6182	10.00	F	3.4	3.1
LT3F6183			1.0	2.2
LT3F6184			0.9	2.8
LT3F6185			-0.6	0.3
LT3F6186			1.6	0.9
		(n)	5	5
		MEANS	1.3	1.9
		SDEVS	1.4	1.2
LT4F6187	1.00	F	-0.9	1.4
LT4F6188			0.5	2.8
LT4F6189			-0.5	1.6
LT4F6190			2.0	3.8
LT4F6191			1.2	1.8
		(n)	5	5
		MEANS	0.5	2.3
		SDEVS	1.2	1.0
LT6F6197	1.00	F	0.3	3.2
LT6F6198			1.0	3.2
LT6F6199			0.8	3.0
LT6F6200			1.3	0.1

NOTE: DATA FOR Dosing phase

DOSAGE IN mg/kg / ANIMAL		SEX	DAY OF PHASE 8	DAY OF PHASE 15
			FEMALE ANIMALS	
LT6F6201	1.00	F	2.3	1.7
		(n)	5	5
		MEANS	1.1	2.2
		SDEVS	0.7	1.4
LT7F6202	10.00	F	0.9	2.6
LT7F6203			1.9	1.3
LT7F6204			0.5	2.1
LT7F6205			1.5	3.3
LT7F6206			2.6	4.4
		(n)	5	5
		MEANS	1.5	2.7
		SDEVS	0.8	1.2

NOTE: DATA FOR Dosing phase

TABLE 4 - **INDIVIDUAL GROSS FINDINGS**

Groups	Control or Test substances	Dose levels mg/kg
1	Negative control: vehicle	0
2	Negative control: aprotinin	1
3		10
4	Positive control: melittin	1
5		10
6	Test substance: PAT protein	1
7		10

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
LT1F6172	F	1/1	24-Jan-02 12:05	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT1F6173	F	1/1	24-Jan-02 12:07	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT1F6174	F	1/1	24-Jan-02 12:07	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT1F6175	F	1/1	24-Jan-02 12:12	24-Jan-02	11	OVARY(IES) Cyst(s), , , Unilateral, ,
LT1F6176	F	1/1	24-Jan-02 12:13	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT2F6177	F	2/1	24-Jan-02 12:14	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT2F6178	F	2/1	24-Jan-02 12:14	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT2F6179	F	2/1	24-Jan-02 12:15	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT2F6180	F	2/1	24-Jan-02 12:15	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT2F6181	F	2/1	24-Jan-02 12:17	24-Jan-02	11	UTERUS Dilatation of horn(s), , Moderate, Bilateral, ,
LT3F6182	F	3/1	24-Jan-02 12:18	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT3F6183	F	3/1	24-Jan-02 12:18	24-Jan-02	11	GENERAL COMMENT All organs, no abnormality, , , , ,
LT3F6184	F	3/1	24-Jan-02 12:19	24-Jan-02	11	UTERUS Dilatation of horn(s), , Moderate, Bilateral, ,
LT3F6185	F	3/1	24-Jan-02 12:20	24-Jan-02	11	GENERAL COMMENT 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
LT3F6186	F	3/1				GENERAL COMMENT
			24-Jan-02 12:20	24-Jan-02	11	All organs, no abnormality, , , , ,
LT4F6187	F	4/1				LIVER
			24-Jan-02 12:21	24-Jan-02	11	Focus(i), white, Left lobe, , Single, ,
LT4F6188	F	4/1				GENERAL COMMENT
			24-Jan-02 12:22	24-Jan-02	11	All organs, no abnormality, , , , ,
LT4F6189	F	4/1				GENERAL COMMENT
			24-Jan-02 12:22	24-Jan-02	11	All organs, no abnormality, , , , ,
LT4F6190	F	4/1				GENERAL COMMENT
			24-Jan-02 12:23	24-Jan-02	11	All organs, no abnormality, , , , ,
LT4F6191	F	4/1				GENERAL COMMENT
			24-Jan-02 12:23	24-Jan-02	11	All organs, no abnormality, , , , ,
LT5F6192	F	5/1				GENERAL COMMENT
			10-Jan-02 14:42	10-Jan-02	98	All organs, no abnormality, , , , ,
LT5F6193	F	5/1				GENERAL COMMENT
			10-Jan-02 14:43	10-Jan-02	98	All organs, no abnormality, , , , ,
LT5F6194	F	5/1				GENERAL COMMENT
			10-Jan-02 12:13	10-Jan-02	98	All organs, no abnormality, , , , ,
LT5F6195	F	5/1				GENERAL COMMENT
			10-Jan-02 14:43	10-Jan-02	98	All organs, no abnormality, , , , ,
LT5F6196	F	5/1				GENERAL COMMENT
			10-Jan-02 11:59	10-Jan-02	98	All organs, no abnormality, , , , ,
LT6F6197	F	6/1				GENERAL COMMENT
			24-Jan-02 12:23	24-Jan-02	11	All organs, no abnormality, , , , ,
LT6F6198	F	6/1				GENERAL COMMENT
			24-Jan-02 12:24	24-Jan-02	11	All organs, no abnormality, , , , ,
LT6F6199	F	6/1				GENERAL COMMENT
			24-Jan-02 12:24	24-Jan-02	11	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
LT6F6200	F	6/1				GENERAL COMMENT
			24-Jan-02 12:25	24-Jan-02	11	All organs, no abnormality, , , , ,
LT6F6201	F	6/1				GENERAL COMMENT
			24-Jan-02 12:25	24-Jan-02	11	All organs, no abnormality, , , , ,
LT7F6202	F	7/1				GENERAL COMMENT
			24-Jan-02 12:25	24-Jan-02	11	All organs, no abnormality, , , , ,
LT7F6203	F	7/1				GENERAL COMMENT
			24-Jan-02 12:26	24-Jan-02	11	All organs, no abnormality, , , , ,
LT7F6204	F	7/1				GENERAL COMMENT
			24-Jan-02 12:26	24-Jan-02	11	All organs, no abnormality, , , , ,
LT7F6205	F	7/1				GENERAL COMMENT
			24-Jan-02 12:27	24-Jan-02	11	All organs, no abnormality, , , , ,
LT7F6206	F	7/1				GENERAL COMMENT
			24-Jan-02 12:27	24-Jan-02	11	All organs, no abnormality, , , , ,

ATTACHMENTS

ATTACHMENT 1 - **PROTOCOL AND AMENDMENTS**

PAT protein: ACUTE TOXICITY BY INTRAVENOUS INJECTION IN THE MOUSE

GENERAL INFORMATION

Study number	:	SA 01352
Test substance	:	PAT (Phosphoacetyl Transferase) protein
Batch number	:	220601701
Biological activity	:	PAT protein confers glufosinate-ammonium tolerance
Storage conditions	:	-20°C

STUDY PERSONNEL

Responsibility		Name
Study Director	:	KENNEL Philippe
In-life Supervisor	:	BONNAFOUS Bernard
Responsible Technician	:	COLOMBEL Sophie

PROPOSED DATES

Arrival of animals	:	January 03, 2002
Experimental starting date	:	January 003, 2002
Acclimatization period in days	:	7
Start of treatment	:	January 10, 2002
First final sacrifice date	:	January 24, 2002
Experimental completion date	:	January 24, 2002

ENVIRONMENTAL CONDITIONS

Animal room number : L4

TREATMENT GROUPS AND DOSAGES

GROUP	CONTROL OR TEST SUBSTANCES	DOSE LEVELS mg/kg	ANIMAL IDENTITY FEMALES	NUMBER OF FEMALE ANIMALS PER GROUP
1	Negative control: vehicle	0	LT1F6172 to 6176	5
2	Negative control:	1	LT2F6177 to 6181	5
3	Aprotinin	10	LT3F6182 to 6186	5
4	Positive control:	1	LTEF6187 to 6191	5
5	Melittin	10	LT5F6192 to 6196	5
6	Test substance:	1	LT6F6197 to 6102	5
7	PAT	10	LT7F6202 to 6206	5

Number of animals in pretest: 42
PathTox number: 01237

Treatment conditions of administration: test substances will be administered at 12 ml/kg and not 10 ml/kg as mentioned in the Master Protocol.

STUDY DIRECTOR:

DATE: December 19, 2001 KENNEL PHILIPPE

SPONSOR:

Aventis CropScience
14-20, rue Pierre Baizet
BP 9163
F-69263 Lyon Cedex 09

SPONSOR REPRESENTATIVE:

DATE: 19 Décembre 2001 HEROUET CORINNE

ACUTE TOXICITY BY INTRAVENOUS INJECTION IN THE MOUSE

TESTING FACILITY:

Aventis CropScience
355, rue Dostoïevski
BP 153
F-06903 Sophia Antipolis Cedex

1 - GENERAL

1.1 PURPOSE OF STUDY

The objective of this study is to investigate the acute toxicity of the test substance (protein) after a single intravenous administration. The potential acute toxicity of the test protein will be compound to the acute intravenous toxicity of two reference proteins: Aprotinin (negative control) and melittin (positive control).

1.2 GOOD LABORATORY PRACTICE COMPLIANCE

This study, with the exception of the test material production, will be performed in accordance with the principles of Good Laboratory Practice ("Bonnes Pratiques de Laboratoire") described in the following issues:

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

-European Commission Directive 1999/11/EC, 1999

-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 (MAFF), notification 12 NohSan N°8628 (December 06, 2000).

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

1.3 QUALITY ASSURANCE

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

2 - OVERVIEW OF THE 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. Mice will be treated by intravenous injection of the test protein in the caudal vein using physiological saline as vehicle.

Animals will be observed for clinical signs and for survival for 15 days. Body weight will be recorded at weekly intervals. Macroscopic observations will be made at the necropsy of mice which die early and at scheduled termination.

3 - SPECIES SPECIFICATIONS

3.1 SPECIES AND STRAIN

Mouse
Ico: OF1 (IOPS Caw)

3.2 ANIMAL SUPPLIER

Iffa Credo (l'Arbresle, France).

3.3 REASON FOR SELECTION OF SPECIES

The mouse has been choosen because of its recommendation by regulatory authorities as an appropriate test species to assess acute intravenous toxicity.

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

3.4 AGE RANGE

Mice will be 7 weeks of age at the start of exposure to the test substance.
They will be acclimatized to laboratory conditions for at least 5 days prior to treatment.

3.5 SELECTION

All animals will be checked on arrival for their health status. The acceptable body weight range will be ± 20% of the mean body weight. Any animal deemed unsuitable for selection is based on weight will not be used in the study and will be maintained as stock animals within the animal facility.

3.6 IDENTIFICATION

Each animal will be identified by a stainless steel ear tag bearing a four-digit animal number.

4 - DIET INFORMATION

4.1 FEED

Certified rodent pellets and irradiated diet AO4C-10 from U.A.R. (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France) will be available *ad libitum*.

4.2 WATER

Filtered and softened water from the municipal water supply will be provided *ad libitum* using water bottle. Filters servicing the watering system are changed regularly and sterilization of the system is periodically performed. For these procedures, animals may be without water for up to 6 hours consecutive during the day of maintenance.

4.3 ANALYSES

Analytical data will be provided by the manufacturer for each batch of diet including the concentrations of nutritional components, selected heavy metals, pesticides, mycotoxins, microorganisms and nitroso compounds. Batches of diet will only be released for use after confirmation they meet specification. Certificates of water analysis are provided by the Laboratoire Municipal d'Hygiène de la Ville de Nice and "Institut d'Hygiène Alimentaire" (Longjumeau).

4.4 RECORDS

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

5 - ENVIRONMENTAL CONDITIONS

5.1 ROOM

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

5.2 CAGING

Animals will be housed individually in suspended, stainless steel, wire mesh cages. The cage of each animal will be identified by a card with a unique identification number, according to the treatment group and dosage.

5.3 TEMPERATURE, HUMIDITY AND VENTILATION

Air temperature will be controlled to ensure:

- a temperature of 20°C - 24°C
- a relative humidity of 40% - 70%

with a target of 15 air changes per hour.

5.4 LIGHTING

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

5.5 RECORDS

The temperature, humidity, lighting and air pressure in the animal room and the performance of the ventilation system are constantly monitored by computer. Records of all deviations from specifications will be placed in the study file.

6 - CONTROL AND TEST SUBSTANCES

6.1 SUBSTANCE CHARACTERISTICS

Negative and positive control substances with known toxicity will be used as reference compounds.

Aprotinin will be used as negative control (a protein with molecular weight 6 500 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 (a protein with molecular weight 2 840 daltons. reference M2272, Sigma-Aldrich, Saint-Quentin Fallavier, France). Melittin is the principle hemolytic component of honey bee venom, highly toxic when administered by intravenous route at 10 mg/kg in mice.

The test substance identification, purity and biological activity will be supplied by the sponsor. Full details of the test substances description will be placed in the study report.

A control group will be administered the vehicle (physiological saline).

6.1.1 Storage conditions

The control and test substances will be stored frozen in an air-tight, light-resistant container at approximately minus 20°C or according to the conditions described in the test substance specifications when available.

6.1.2 Safety handling and requirements

Information on the appropriate safety precautions when handling control and test substances will be given by the supplier and the sponsor. In the absence of information on the potential toxic effects of the test substance, safety precautions will be applied according to the relevant standard operating procedures.

6.1.3 Analyses

Identity, purity and stability of the control and test substances will be provided by the supplier and the Sponsor Representative, respectively.

6.2 TEST SUBSTANCE FORMULATION

6.2.1 Preparation

For each dose level, the appropriate amount of control and test substances will be dissolved in sterilized physiological saline. The formulations will be prepared and used on the day of dosing. The unused residues of formulations will be discarded at the end of the administration period.

6.2.2 Storage conditions

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

6.2.3 Analyses

No analyses will be performed on the test formulations.

7 - TREATMENT GROUPS AND DOSAGES

7.1 CHOICE OF DOSES

The dose levels were set after discussion with the sponsor and based on the typical acute toxicity of reference compounds.

7.2 CHOICE OF ROUTE

The intravenous route was selected to ensure maximum exposure to the test substance.

7.3 CONDITIONS OF ADMINISTRATION

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

8 - LABORATORY DETERMINATIONS AND SCHEDULES

8.1 CLINICAL EXAMINATION

8.1.1 Clinical signs and mortality

Clinical signs will be recorded starting on Day 1 and every day through Day 15. They will be recorded approximately 1 hour after dosing, at least once more on Day 1 and at least once daily thereafter. 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 mortality (once daily except on week-ends and public holidays). Any animal suffering from severe distress, in a moribund condition or considered unlikely to survive will be sent for early necropsy.

8.1.2 Body weight

Body weights will be measured at least twice during the acclimatization phase, on study Days 1, 8 and 15 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 at the earliest opportunity.

Terminal necropsy and animals *in extremis:*
Animals will be anesthetized by intraperitoneal injection of pentobarbital, then exsanguinated under deep anesthesia before necropsy.

8.2.2 Necropsy

The necropsy of animals will include macroscopic examination of abdominal and thoracic cavities, major organs and tissues.

Significant macroscopic findings will be recorded. Tissues may be sampled at the discretion of the Pathologist.

9 - CALCULATIONS

For body weights, means and standard deviations will be calculated.

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 in the final report. The final report will include the information and data required by current internationally recognized regulations.

11 - ARCHIVING

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

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

Aventis CropScience
355, rue Dostoïevski
BP 153
F-06903 Sophia Antipolis Cedex

PROTOCOL AMENDMENT

Protocol SA 01352

PAT (Phosphoacetyl Transferase) protein

Acute toxicity by intravenous injection in the mouse

Protocol amendment: N° 1

Reason(s):

Environmental conditions
- Room
The animal room number will be L8 and not L4.
- Records
Air pressure in the animal room will not be constantly monitored by computer.

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

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

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

Study Director: KENNEL Philippe Date: January 07, 2002

PROTOCOL AMENDMENT

Protocol SA 01352

PAT (Phosphoacetyl Transferase) protein

Acute toxicity by intravenous injection in the mouse

Protocol amendment: N° 2

Reason(s):

Treatment groups en dosages
Animal identity in group 4 should have been written "LT4F6187 to 6191" and not "LTEF6187 to 6191".

Study Director: KENNEL Philippe Date: January 11, 2002

PROTOCOL AMENDMENT

Protocol SA 01352

PAT (Phosphoacetyl Transferase) protein

Acute toxicity by intravenous injection in the mouse

Protocol amendment: N° 3

Reason(s): Typing error.

1 - GENERAL

1.1 PURPOSE OF STUDY

The word "compound" should have been written "compared".

Study Director: KENNEL Philippe

Date: February 05, 2002

ATTACHMENT 2 - **GLP COMPLIANCE CERTIFICATE**

ATT. 2

REPUBLIQUE FRANCAISE

PREMIER MINISTRE

GROUPE INTERMINISTERIEL DES PRODUITS CHIMIQUES

LE PRESIDENT

Tél : 01 53 44 97 58

n° 67

Paris, le 6 NOV. 2000

La correspondance est à adresser aux :
Secrétariat du GIPC
Le BERVIL
12 rue Villiot
75572 PARIS CEDEX 12

OBJET : Evaluation de la conformité aux B.P.L. selon la directive 88/320/C.E.E.

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, 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 Essais a décidé pour votre installation d'essai du statut suivant :

respect des principes de B.P.L.
Domaines de reconnaissance :
- ☐ 1 - **essais physico-chimiques**
- ☐ 2 - **études de toxicité**
- ☐ 3 - **études de mutagénicité**
- ☐ 4 - **études écotoxicologiques sur les organismes aquatiques et terrestres**
- ☐ 5 - études portant sur le comportement dans l'eau, dans le sol et dans l'air ; bioaccumulation
- ☐ 6 - études portant sur les résidus -
- ☐ 7 - études portant sur les effets sur les mésocosmes et les écosystèmes naturels
- ☐ 8 - **méthodes de chimie analytique et clinique**
- ☐ 9 - **métabolisme animal**

Date d'inspection : 3- 4 juillet 2000

- ☐ inspection initiale (i.i)
- ☐ inspection périodique (i.p)
- ☐ inspection complémentaire (i.c)
- ☐ inspection d'extension (i.e)
- ☐ inspection de renouvellement (i.r)

Date de décision du G.I.P.C. : 5 octobre 2000
Date de prise d'effet : 4 juillet 2000
Année de première conformité : 1992
Date de validité : 5 avril 2002

Le Conseiller d'Etat h.
Président du Groupe Interministériel des Produits Chimiques

M. Pierre CREYSSEL

Aventis Crop Science
BP 153
06903 SOPHIA ANTIPOLIS CEDEX

SA
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ATTACHMENT 3 - **CERTIFICATES OF ANALYSIS**

Sigma-Aldrich Certificate of Analysis

Product Number:**A4529**

Product Name:**Aprotinin**

SIGMA

Certificate of Analysis

TEST	SPECIFICATION	LOT {080K7022} RESULTS
Product Name	Aprotinin	
Product Number	A4529	
CAS Number	9087701	
APPEARANCE	REPORT RESULT	OFF-WHITE POWDER WITH LIGHT TAN CAST
ACTIVITY	3 TO 7 TIU/MG SOLID	4.8 TIU/MG SOLID
UNIT DEFINITION	ONE TRYPSIN INHIBITOR UNIT (TIU) WILL DECREASE THE ACTIVITY OF 2 TRYPSIN UNITS BY 50% WHERE ONE TRYPSIN UNIT WILL HYDROLYZE 1.0 MICROMOLE OF N-ALPHA-BENZOYL-DL-ARGININE P-NITROANILIDE (BAPNA) PER MINUTE AT PH 7.8 AT 25 DEG C.	
QC ACCEPTANCE DATE		SEPTEMBER 2000

David Feldker, Manager
Analytical Services

COPIE CERTIFIÉE CONFORME
LE 22/04/02 VISA CFA

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Att. 3

Sigma-Aldrich Certificate of Analysis

Product Number:**M2272**

Product Name:**Melittin**

SIGMA

Certificate of Analysis

TEST	SPECIFICATION	LOT {021K4008} RESULTS
Product Name	Melittin	
Product Number	M2272	
CAS Number	37231280	
APPEARANCE	WHITE TO FAINT YELLOW WITH A FAINT TAN CAST POWDER	FAINT YELLOW POWDER WITH A FAINT TAN CAST
SOLUBILITY	CLEAR YELLOW TO YELLOW-TAN SOLUTION AT 50 MG/ML IN WATER	CLEAR LIGHT YELLOW-TAN
PHOSPHOLIPASE A2 CONTENT	LESS THAN 5 UNITS/MG SOLID	<1 UNIT/MG SOLID
PURITY BY HPLC	MINIMUM 85%	93%
QC ACCEPTANCE DATE		MARCH 2001

David Feldker, Manager
Analytical Services

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LE 22/01/02 VISA CFA

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Att. 3

Aventis CropScience

Aventis

CERTIFICATE OF ANALYSIS

Aventis CropScience N.V.
Biotech Product Characterization
Jozef Plateaustraat 22
B-9000 GENT - BELGIUM
Tel : +32 (0)9 235 8430 Fax : +32 (0)9 224 0694

No. : EC-02-LL003

COPIE CERTIFIÉE CONFORME
LE 04/12/02 VISA

Origin of the Certified Material
Aventis CropScience N.V.
Jozef Plateaustraat 22
B-9000 GENT- BELGIUM

General Protein Information

- Product name	:	PAT protein (*bar* gene)
- Batch number	:	220601701
- Concentration	:	as determined with ELISA = 0.848 mg/ml
- Buffer	:	20 mM Tris/HCl pH = 7.5 1 mM DTT 5 mM EDTA 100 mM NaCl
- Endotoxins	:	protein material derived in *E. Coli.* as determined with Biowittaker KQCL-test < 0.00375 EU/µg
- SDS-PAGE	:	estimated purity : > 95% (see Attachment I for scan)
- Total amount	:	3 aliquots 2 aliquots containing 1.00 ml = 1.696 mg 1 aliquot containing 0.550 ml = 0.467 mg 2.162 mg
- Storage	:	-70°C till use
- Handling	:	work endotoxin-free - wear gloves - heat glass at 180°C or use LPS-free disposable bottles - soak spatulas, stirringfleas,.... In 0.5M NaOH - use new pots of chemicals - use LPS-free water - use LPS-free safe-lock tubes, tips (Eppendorf Biopur)
- Attachments	:	1

Responsible Scientist

04.12.01

Liesbeth Lambert, date

Teamleader of Biotech Product Characterization

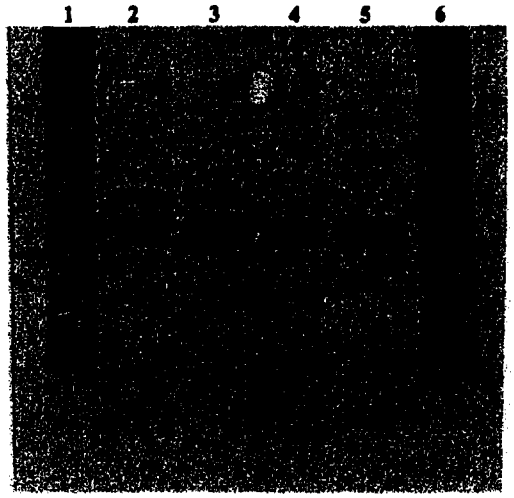
04.12.01

Marc Van den Bulcke, date

Aventis CropScience

Aventis

Attachment I



Legend :

1.	MW Marker	
2.	22060170	loaded 2.5µg = startmaterial
3.	22060170	loaded 5µg = startmaterial
4.	220601701	loaded 2.5µg = purified material
5.	220601701	loaded 5µg = purified material
6.	MW Marker	

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: Aventis CropScience

Company Representative:

Title:

Signature:

Date: _____

These data are the property of Aventis 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.

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

Bayer CropScience

DOCMAP N°605512

PAT (PHOSPHINOTHRICIN ACETYLTRANSFERASE) PROTEIN DERIVED FROM *bar* GENE

ACUTE TOXICITY BY INTRAVENOUS INJECTION IN THE MOUSE

REPORT OF STUDY SA 01352

AMENDED REPORT

STUDY DIRECTOR: P. KENNEL

PERFORMING LABORATORY:

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

SPONSOR:

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

STUDY COMPLETED ON: MARCH 13; 2002
AMENDED ON: AUGUST 29, 2002

FINAL REPORT AMENDMENT

Amendment N°1

Reasons: 1- The name of the test substance is PAT (Phosphinothricin AcetylTransferase) protein and not PAT (Phosphoacetyl Transferase) as mentioned in all the study file including raw data, protocol and report.

2- Acquisition of Aventis CropScience by Bayer.

- The company name is changed to Bayer CropScience.
- The new Sponsor address is:

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

Changes were audited on August 29, 2002.

Quality Assurance Group Leader

Date: August 29, 2002

G. ODAGLIA

Study Director:

Date: August 29, 2002

P. KENNEL