Annex 2.3 :

Sensitizing and irritation studies

Richeux F. (2002)
Assessment of sensitising properties on albino guinea pig, maximisation test according to Magnusson and Kligman.
Phycher Bio Développement, report n° SMK-PH-02/0051.

Richeux F. (2002)
Assessment of acute irritant/ corrosive effect on the skin,
Phycher Bio Développement, report n°IC-OCDE-PH-02/0051.

Richeux F. (2002)
Assessment of acute irritant/ corrosive effect on the eyes,
Phycher Bio Développement, report n°IO-OCDE-PH-02/0051.

Phycher

Bio développement

ACCORDING TO THE G.L.P.
DESCRIBED IN 88/320 E.E.C. AND
C/81/130 O.E.C.D. DIRECTIVES
BY G.I.P.C. 10/23/98

Assessment of sensitising properties on albino guinea pig

Maximisation test according to Magnusson and Kligman

Final Report: SMK-PH-02/0051

TEST PRODUCT
Mannostab^{MD}
Batch n°10064/06-2000

❑ **STUDY MONITOR** : **ADEC TOX**
[redacted]
120, rue Quintin
F-33000 BORDEAUX

❑ **SPONSOR** : **LAFFORT OENOLOGIE**
Quai de Souys
BP 17
F-33015 BORDEAUX Cedex

Report of 17 pages and 2 appendices **Cestas, June 18th, 2002**

CONTENTS

PART 1

PART 2

QUALITY ASSURANCE ATTESTATION

I, the undersigned Bernard Benech, Quality Assurance unit of PHYCHER *Bio développement*, attest that the study **SMK-PH-02/0051** was submitted to the inspection of Quality Assurance.

The routine inspections of the toxicity tests performed within the laboratory of PHYCHER *Bio développement*, are carried out as a control process of different main technical phases concerning at least one similar test. The frequency is once a term or more. Dates of technical phases inspections relating to this test are given below :

Date of inspection	: March 26th, 2002 *Preliminary studies: MNNC and Pre-MNIC determinations*
Date of reporting to Study Director	: March 26th, 2002
Date of reporting to Management	: March 26th, 2002

This report has been audited by PHYCHER *Bio développement* Quality Assurance unit. It is considered to be an accurate account of the data generated and the application of the operating procedures in use within the laboratory :

	Draft report	Final report
Date of audit report inspection	: May 03rd, 2002	June 18th, 2002
Date of reporting to Study Director	: May 03rd, 2002	June 18th, 2002
Date of reporting to Management	: May 03rd, 2002	June 18th, 2002

Date June 18th, 2002

[redacted]

AUTHENTICATION

I, the undersigned, François Richeux, Study director, certify that the study **SMK-PH-02/0051** was performed on the premises of the Laboratory PHYCHER *Bio développement* under the technical responsibility of Corinne Scheyder.

I, certify that the objectives laid down in the technical protocol were achieved and no undesirable event occurred to affect the quality or the integrity of the study. I consider the data generated to be valid. This report fully and accurately reflects the operating procedures used and data generated.

The entire work was performed in compliance with the principles of the Good Laboratory Practices (G.L.P.), as defined in *the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) Appendix 2 - May 12th, 1981; C (97) 186, November 26th, 1997)*, and transcribed in the *decree n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française.*

Date June 18th, 2002

[redacted]

Study director

SUMMARY AND CONCLUSION OF THE STUDY

After induction (intradermic injection and topical application) of 20 animals (female) of treated group with the test product **Mannostab**[MD] and a 18-days rest phase, the 1st challenge phase, under occlusive dressing for 24 hours, consisted to a single topical application of the test product diluted at 50% and 25% in distilled water, according to the experimental protocol established *from the O.E.C.D. guideline n°406 dated July 17th,1992 and the method B.6 of the E.E.C. n°96/54 dated July 30th, 1996.*

It was noted a macroscopic cutaneous reaction (moderate erythema) in 5% of treated animals (1/20), 24 and 48 hours following the removal of the occlusive dressing, on the area treated at 50%.
No cutaneous intolerance reaction was recorded as in animals from the negative control group than in animals from the treated group at 25%.

In view to confirm or infirm this reaction, a second challenge phase has been carried out after a 11-days rest phase with the test product diluted at 10% and 5% in distilled water.

No macroscopic cutaneous reactions attributable to allergy were recorded during the examination following the removal of the occlusive dressing.

In conclusion, in view of these results, under these experimental conditions, the product **Mannostab**[MD], and in accordance with the criteria for classification, packaging and labelling of dangerous substances of the E.E.C. Directives 67/548 and 93/21, **must not be classified.**

Date June 18th 2002

Scientific Direction PHYCHER *Bio développement*

[redacted]

Pharmacologist
(Hdr)

cofrac
ESSAIS
ACCREDITATION
N° 1-0887

TEST REPORT

1 - TEST PRODUCT

The test product **Mannostab**[MD], sent by ADEC TOX - 120 rue Quintin – F-33000 Bordeaux FRANCE, was received on 02/20/02. Its characteristics were:

- Container : plastic pilular (n=3)
- Quantity : 80.97g (container + contents)
- Batch : 10064/06-2000
- Form : powder
- Colour : whitish
- Storage : room temperature

It was identified under the code number: **PH-02/0051**.

Informations concerning the identity, purity and stability of the test product are the responsibility of the Sponsor. An information data sheet concerning the product was provided by the study monitor.

2 - METHODOLOGY

The test was carried out between 02/25/2002 and 04/18/2002, in *PHYCHER Bio développement - ZI de Toctoucau - 18 chemin de Lou Tribail - 33611 Cestas Cedex*, according to the protocol *Ref. SMK-version February 2002,* established according to *the Magnusson and Kligman method (J. Invest. Dermatol. 1969. 52, 268-276) and in accordance with O.E.C.D. Guideline N° 406 of July 17th, 1992, and the test method B.6 of the 96/54 E.E.C. Directive.*

2.1 - Animals

30 albino guinea pigs of Dunkin-Hartley strain, supplied by Centre de Production Animale (F-45160 Olivet) were exposed to the test product after a 5-day acclimatisation period. For the main study, the animals weighted between 303g and 414g at the beginning of the test.

2.1.1 - Preliminary studies

Maximum Non Necrotizing Concentration (M.N.N.C.) determination :
2 female guinea pigs identified C6393 and C6394.

The test product was injected by intradermal route at the following concentrations: 40%, 30%, 25%, 12.5%, 6.25%, 3.125%, 1.5625% and 0.781% diluted in a physiological saline solution.

Pre-Maximum Non Irritant Concentration (M.N.I.C.) determination :
2 female guinea pigs identified C6393 and C6394.

The product was applied under an occlusive dressing during 24 hours, at the following concentrations pure (100%) and diluted 50%, 25% and 12.5% in a physiological saline solution.

Maximum Non Irritant Concentration (M.N.I.C.) determination :
3 female guinea pigs identified C6401 to C6403

After induction by intradermal injection with physiological saline solution and by topical application with distilled water and a 17-days rest phase, the challenge phase under occlusive dressing for 24 hours consists in a single topical application of the test product at the following concentrations 100%, diluted 50%, 25% and 12.5% in distilled water

2.1.2 - Main study

GROUP 1 (negative control) : 10 female guinea pigs identified C6473 to C6482;
GROUP 2 (treated) : 20 female guinea pigs identified C6483 to C6502;

Note : The results of the 3 latest positive group (Reference substance : neomycin sulfate Test 6 and benzocaïne Test 4 and 5) carried out as method sensibility, were presented in appendix.

The environmental parameters were :
- Temperature : between 20 °C and 25 °C
- Relative humidity : between 32% and 59%

2.2 - Chronological development

➢ *Preliminary studies*

02/21/02	Arrival of animals.
02/25/02 to 03/27/02	Preliminary tests : pre MNIC, MNNC, and MNIC determinations.

➢ *Main study*

02/28/02	Arrival of animals. Induction phase 1st induction
03/05/02	- 2 intradermal injections of the product diluted at 40% in a physiological saline solution. - 2 intradermal injections of Freund's Complete Adjuvant diluted at 50% in a physiological saline solution. - 2 intradermal injections of a mixture with equal volumes - Freund's Complete Adjuvant at 50% and the product diluted at 80% in a physiological saline solution.
03/05/02	Weighing of animals.
03/13/02	2nd induction: topical application, on the same zone, with the product at 100%, 24 hours after brushing with 0.5 ml of a solution of Sodium lauryl sulfate at 10%.
03/15/02 to 04/02/02	Rest phase: 18 days
04/02/02	1st Challenge phase : topical application under occlusive dressing at the following concentrations : 50% and 25%.
04/04/02	24-hours reading time.
04/05/02	48-hours reading time and weighing.
04/04/02 to 04/15/02	Rest phase: 11 days
04/15/02	2nd Challenge phase : topical application under occlusive dressing at the following concentrations : 10% and 5%.
04/17/02	24-hours reading time.
04/18/02	48-hours reading time and weighing.

3 - RESULTS

3.1 - Concentrations selected

Preliminary studies :

- *MNNC determination :*

No necrosis has been observed since the concentrations of 40%, the first induction has been carried out by intradermal injection at the same concentration (*table 1*, page 9).

-*Pre MNIC determination :*

24 hours after the removal of the occlusive dressings, no macroscopic cutaneous reaction was recorded (*table 2*, page 10).
In view of these results, the concentrations selected were pure for the 2nd induction of the main study and the MNIC began at the concentration of 100%

- *MNIC determination :*

24 hours after removal of the occlusive dressings, it was noted a slight erythema, on the treated area at 100% in 2 animals (*table 3*, page 10).
In view of this result, the concentrations selected were 50% (MNIC) and 25% (1/2 MNIC) for the challenge phase.

Main study :

- *induction phase :*

The induction phase was performed by intradermal injection at D0 with the test product diluted at 40% and by topical application at D7 with the test product at 100%, after brushing with a solution of sodium lauryl sulfate, as planned *in the part 4.4.2.1 of the experimental protocol.*

- *1st challenge phase :*

The test product has been used diluted at 50% and diluted at 25% (1/2 MNIC) as planned *in the part 4.4.4 of the experimental protocol.*

- *2nd challenge phase :*

The test product has been used diluted at 10% and diluted at 5% as planned *in the part 5 of the experimental protocol.*

3.2 - Sensitising potential assessment

Overall results of the 1st challenge phase (readings at 24 and 48 hours) are given *in table 4* page 11.

Individual scores of macroscopic evaluations performed during 1st challenge phase are given in *table 5* page 12.

It was noted a macroscopic cutaneous reaction (moderate erythema) in 5% of treated animals (1/20), 24 and 48 hours following the removal of the occlusive dressing, on the area treated at 50%.
No cutaneous intolerance reaction was recorded as in animals from the negative control group than in animals from the treated group at 25%.

In view to confirm or infirm this reaction, a second challenge phase has been carried out after a 11-days rest phase with the test product diluted at 10% and 5% in distilled water.

Overall results of the 2nd challenge phase (readings at 24 and 48 hours) are given *in table 6* page 13.

Individual scores of macroscopic evaluations performed during 2nd challenge phase are given in *table 7* page 14.

No macroscopic cutaneous reactions attributable to allergy were recorded during the examination following the removal of the occlusive dressing.

3.3 - Weight evolution

The weight growth of negative control animals (Group 1) and treated animals (Group 2) is respectively presented in *tables 8 and 9* (pages 15 and 16).

Not any abnormality was recorded in the weight growth of both groups.

4 - CONCLUSION

In view of these results, under these experimental conditions, the product **Mannostab**[MD], in accordance with the criteria for classification, packaging and labelling of dangerous substances of the E.E.C. Directives 67/548 and 93/21, **must not be classified**.

5 - ARCHIVES

All the original data related and the final report related to this study will be stored for a period of 10 years in the company under the reference **SMK-PH-02/0051**. After this period, the Sponsor's instructions will be applied.

6 - PROTOCOL ADHERENCE

No deviation was recorded during the study.

Date 04/18/02.

[redacted]

SENSITISATION STUDY: PRELIMINARY STUDY

TEST PRODUCT: Mannostab[MD]

Table 1

MNNC DETERMINATION

Macroscopic evaluation of cutaneous reactions

Date injection : 02/25/02 & 02/28/02
Animals : 2

Operator : [redacted]

Injection	Animals	CONCENTRATIONS							
		40%	30%	25%	12.5%	6.25%	3.125%	1.562%	0.781%
Intradermic injection	C6393	2	0	0	0	0	0	0	0
	C6397	2	0	0	0	0	0	0	0

Maximal Non Necrotizing Concentration	**MNNC**	**40%**

SENSITISATION STUDY: PRELIMINARY STUDY

TEST PRODUCT: Mannostab[MD]

Table 2

Pre-MNIC DETERMINATION

Macroscopic evaluation of cutaneous reactions

Application date (D0): 02/25/02
Animals : 2

Operator : [redacted]

APPLICATION	Animals	CONCENTRATIONS			
		100%	50%	25%	12.5%
Topical application under occlusive dressing	C6393	0	0	0	0
	C6394	0	0	0	0

Table 3

MNIC DETERMINATION

Macroscopic evaluation of cutaneous reactions

Application date (D0): 02/27/02
Animals : 3

Operator : [redacted]

APPLICATION	N° OF ANIMALS	CONCENTRATIONS			
		100%	50%	25%	12.5%
Topical application under occlusive dressing	C6401	0	0	0	0
	C6402	1	0	0	0
	C6403	1	0	0	0

Maximal Non Irritant Concentration	**MNIC**	**50%**

SENSITISATION STUDY: MAIN STUDY

TEST PRODUCT: Mannostab^{MD}

Application : topical under occlusive dressing

Operator : [redacted]

Application date (D0) : 03/05/2002

Animals : 30
- Negative control group : 10
- Treated group : 20

Table 4

ASSESSMENT OF THE SENSITISING POTENTIAL
1st challenge

Overall results
Macroscopic evaluation (readings at 24 and 48 hours) of cutaneous reactions

GROUPS	Reading time	Concen-trations	COTATIONS				% of positive responses
			0	1	2	3 or >	
NEGATIVE CONTROL GROUP	24 h	50%	9	1	0	0	0%
	48 h	50%	10	0	0	0	0%
	24 h	25%	10	0	0	0	0%
	48 h	25%	10	0	0	0	0%
TREATED GROUP	24 h	50%	19	0	1	0	5%
	48 h	50%	17	2	1	0	5%
	24 h	25%	20	0	0	0	0%
	48 h	25%	20	0	0	0	0%

SENSITISATION STUDY: MAIN STUDY

TEST PRODUCT: Mannostab[MD]

Application : topical under occlusive dressing

Operator : [redacted]

Application date (D0) : 03/05/02

Animals : 30
- Negative control group : 10
- Treated group : 20

Table 5

ASSESSMENT OF THE SENSITISING POTENTIAL
1st challenge

Individual results
Macroscopic evaluation (readings at 24 and 48 hours) of cutaneous reactions

N° of Animals		M.N.I.C. (50%)				½ M.N.I.C. (25%)			
		24 hours		48 hours		24 hours		48 hours	
NEGATIVE CONTROL GROUP *(Distilled water)*		Er	Oe	Er	Oe	Er	Oe	Er	Oe
N° C 6473	F	0	0	0	0	0	0	0	0
N° C 6474	F	0	0	0	0	0	0	0	0
N° C 6475	F	0	0	0	0	0	0	0	0
N° C 6476	F	0	0	0	0	0	0	0	0
N° C 6477	F	0	0	0	0	0	0	0	0
N° C 6478	F	0	0	0	0	0	0	0	0
N° C 6479	F	1	0	0	0	0	0	0	0
N° C 6480	F	0	0	0	0	0	0	0	0
N° C 6481	F	0	0	0	0	0	0	0	0
N° C 6482	F	0	0	0	0	0	0	0	0
TREATED GROUP *(Test product)*		Er	Oe	Er	Oe	Er	Oe	Er	Oe
N° C 6483	F	0	0	0	0	0	0	0	0
N° C 6484	F	0	0	0	0	0	0	0	0
N° C 6485	F	0	0	0	0	0	0	0	0
N° C 6486	F	2	0	2	0	0	0	0	0
N° C 6487	F	0	0	1	0	0	0	0	0
N° C 6488	F	0	0	0	0	0	0	0	0
N° C 6489	F	0	0	0	0	0	0	0	0
N° C 6490	F	0	0	0	0	0	0	0	0
N° C 6491	F	0	0	0	0	0	0	0	0
N° C 6492	F	0	0	0	0	0	0	0	0
N° C 6493	F	0	0	0	0	0	0	0	0
N° C 6494	F	0	0	0	0	0	0	0	0
N° C 6495	F	0	0	0	0	0	0	0	0
N° C 6496	F	0	0	0	0	0	0	0	0
N° C 6497	F	0	0	1	0	0	0	0	0
N° C 6498	F	0	0	0	0	0	0	0	0
N° C 6499	F	0	0	0	0	0	0	0	0
N° C 6500	F	0	0	0	0	0	0	0	0
N° C 6501	F	0	0	0	0	0	0	0	0
N° C 6502	F	0	0	0	0	0	0	0	0

Er : Erythema Oe : Oedema F: female

SENSITISATION STUDY: MAIN STUDY

TEST PRODUCT: Mannostab^{MD}

Application : topical under occlusive dressing

Operator : [redacted]

Application date (D0) : 03/05/2002

Animals : 30
- Negative control group : 10
- Treated group : 20

Table 6

ASSESSMENT OF THE SENSITISING POTENTIAL
2nd challenge

Overall results
Macroscopic evaluation (readings at 24 and 48 hours) of cutaneous reactions

GROUPS	Reading time	Concen-trations	COTATIONS				% of positive responses
			0	1	2	3 or >	
NEGATIVE CONTROL GROUP	24 h	10%	10	0	0	0	0%
	48 h	10%	10	0	0	0	0%
	24 h	5%	10	0	0	0	0%
	48 h	5%	10	0	0	0	0%
TREATED GROUP	24 h	10%	20	0	0	0	0%
	48 h	10%	20	0	0	0	0%
	24 h	5%	20	0	0	0	0%
	48 h	5%	20	0	0	0	0%

SENSITISATION STUDY: MAIN STUDY

TEST PRODUCT: Mannostab[MD]

Application : topical under occlusive dressing

Operator : [redacted]

Application date (D0) : 03/05/02

Animals : 30
- Negative control group : 10
- Treated group : 20

Table 7

ASSESSMENT OF THE SENSITISING POTENTIAL
2nd challenge

Individual results
Macroscopic evaluation (readings at 24 and 48 hours) of cutaneous reactions

N° of Animals		10%				5%			
		24 hours		48 hours		24 hours		48 hours	
NEGATIVE CONTROL GROUP *(Distilled water)*		Er	Oe	Er	Oe	Er	Oe	Er	Oe
N° C 6473	F	0	0	0	0	0	0	0	0
N° C 6474	F	0	0	0	0	0	0	0	0
N° C 6475	F	0	0	0	0	0	0	0	0
N° C 6476	F	0	0	0	0	0	0	0	0
N° C 6477	F	0	0	0	0	0	0	0	0
N° C 6478	F	0	0	0	0	0	0	0	0
N° C 6479	F	0	0	0	0	0	0	0	0
N° C 6480	F	0	0	0	0	0	0	0	0
N° C 6481	F	0	0	0	0	0	0	0	0
N° C 6482	F	0	0	0	0	0	0	0	0
TREATED GROUP *(Test product)*		Er	Oe	Er	Oe	Er	Oe	Er	Oe
N° C 6483	F	0	0	0	0	0	0	0	0
N° C 6484	F	0	0	0	0	0	0	0	0
N° C 6485	F	0	0	0	0	0	0	0	0
N° C 6486	F	0	0	0	0	0	0	0	0
N° C 6487	F	0	0	0	0	0	0	0	0
N° C 6488	F	0	0	0	0	0	0	0	0
N° C 6489	F	0	0	0	0	0	0	0	0
N° C 6490	F	0	0	0	0	0	0	0	0
N° C 6491	F	0	0	0	0	0	0	0	0
N° C 6492	F	0	0	0	0	0	0	0	0
N° C 6493	F	0	0	0	0	0	0	0	0
N° C 6494	F	0	0	0	0	0	0	0	0
N° C 6495	F	0	0	0	0	0	0	0	0
N° C 6496	F	0	0	0	0	0	0	0	0
N° C 6497	F	0	0	0	0	0	0	0	0
N° C 6498	F	0	0	0	0	0	0	0	0
N° C 6499	F	0	0	0	0	0	0	0	0
N° C 6500	F	0	0	0	0	0	0	0	0
N° C 6501	F	0	0	0	0	0	0	0	0
N° C 6502	F	0	0	0	0	0	0	0	0

Er : Erythema Oe : Oedema F: female

SENSITISATION STUDY: MAIN STUDY

TEST PRODUCT: Mannostab[MD]

Application: topical under occlusive dressing

Operator: [redacted]

Application date (D0): 03/05/02

Animals: 10

Table 8

WEIGHT EVOLUTION
(In grams)

GROUP 1 – NEGATIVE CONTROL

ANIMALS	SEX	Beginning of the test	End of the test
N°C 6473	F	408	829
N°C 6474	F	414	672
N°C 6475	F	408	595
N°C 6476	F	396	611
N°C 6477	F	395	728
N°C 6478	F	378	546
N°C 6479	F	404	628
N°C 6480	F	339	595
N°C 6481	F	338	663
N°C 6482	F	325	660
MEAN		**380.5**	**652.7**
Standard-deviation		**33.8**	**80.1**

F: female

SENSITISATION STUDY: MAIN STUDY

TEST PRODUCT: Mannostab[MD]

Application: topical under occlusive dressing

Operator: [redacted]

Application date (D0): 03/05/02

Animals: 20

Table 9

WEIGHT EVOLUTION
(In grams)

GROUP 2 - TREATED

ANIMALS	SEX	Beginning of the test	End of the test
N°C 6483	F	376	605
N°C 6484	F	313	593
N°C 6485	F	303	595
N°C 6486	F	306	508
N°C 6487	F	344	526
N°C 6488	F	357	628
N°C 6489	F	371	600
N°C 6490	F	362	677
N°C 6491	F	367	659
N°C 6492	F	339	631
N°C 6493	F	384	633
N°C 6494	F	383	573
N°C 6495	F	361	661
N°C 6496	F	365	635
N°C 6497	F	340	595
N°C 6498	F	370	644
N°C 6499	F	319	558
N°C 6500	F	389	562
N°C 6501	F	357	628
N°C 6502	F	369	688
MEAN		**353.8**	**610.0**
Standard deviation		**26.1**	**47.9**

F: female

APPENDICES

Results of positive control (1 page)

Animals diet sheet (1 page)

Phycher

Bio développement

SENSITIZATION STUDY: Positive Control

TEST SUBSTANCES : BENZOCAINE (4th & 5th test)
NEOMYCIN SULFATE (6th test)

Application date (test 4) : 03/14/00 Animals : 10
Application date (test 5) : 02/20/01 Animals : 10
Application date (test 6) : 01/15/02 Animals : 10

Overall results
Macroscopic evaluations (reading at 24 and 48 hours) of cutaneous reactions

Test	Reading Time	Concentrations	COTATIONS				% of Positive Responses
			0	1	2	3	
TREATED GROUP 4th test	24 h	25%	1	4	5	0	50%
	48 h	25%	3	2	5	0	50%
	24 h	12.5%	1	4	5	0	50%
	48 h	12.5%	3	2	5	0	50%
TREATED GROUP 5th test	24 h	12.5%	1	5	4	0	40%
	48 h	12.5%	2	3	5	0	50%
	24 h	6.25%	1	5	4	0	40%
	48 h	6.25%	2	3	5	0	50%
TREATED GROUP 6th test	24 h	75%	4	2	4	0	40 %
	48 h	75%	5	1	4	0	40 %
	24 h	38%	4	3	3	0	30 %
	48 h	38%	4	2	4	0	40 %

In conclusion, in view of these results, under these experimental conditions, the substances **NEOMYCIN SULFATE and BENZOCAINE** :

must be classified **R 43 "may cause sensitization by skin contact"** and in accordance with the criteria for classification, packaging and labelling of dangerous substances of *the E.E.C. Directives 67/548 and 93/21.*

[redacted]

Siège social : 18, chemin de Lou Tribail - ZI de Toctoucau - 33611 CESTAS Cedex - ✆ 05 57 97 16 80 - Fax 05 57 97 16 81

S.A.R.L. AU CAPITAL DE 60 979.61 Euros APE 742C - SIRET 402 924 088 00014

GUINEA PIG BREEDING DIETS

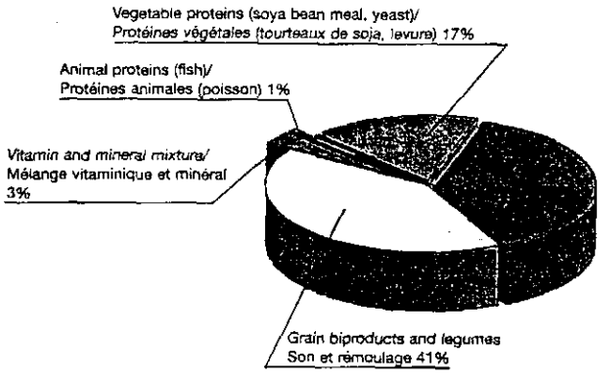
COBAYES - ELEVAGE

Certified	*: 114*
Irradiated Certified	*: 114-10*
Control	*: R14C*
Control Irradiated	*: R14C-10*
Form	*: Pellets ø 3 mm*
Standard pack	
- Certified	*: 20 Kg paper bag*
- Control or irradiated	*: packing case of 2 x 10 Kg vaccum package*
Rate per day	*: 35 to 50 g.*

Certifié	: 114
Certifié irradié	: 114-10
Contrôle	: R14C
Contrôle irradié	: R14C-10
Présentation	*: Pellets ø 3 mm*
Contionnement	
- Certifié	: 20 Kg en sac papier
- Contrôle ou irradié	: carton de 2 x 10 Kg sous vide
Ration journalière	: 35 à 50 g.

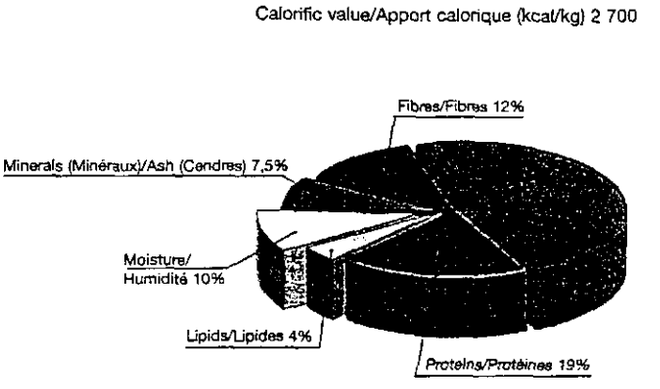
Formula

Formule



Average analysis

Analyse moyenne



Amino acid values (calculated/kg)

Apport en acides aminés (calculé/Kg)

13 000 mg	Arginine
3 000 mg	Cystine
8 700 mg	Lysine
2 800 mg	Methionine
2 100 mg	Tryptophan
8 100 mg	Glycine

Fatty acid values (calculated/kg)

Apport en acides gras (calculé/Kg)

6 600 mg	palmitic ac.
200 mg	palmitoleic ac.
2 700 mg	stearic ac.
10 500 mg	oleic ac.
12 800 mg	linoleic ac.
3 000 mg	linolenic ac.

Mineral and vitamin content

Contenu en vitamines et minéraux

MINERALS calculated/kg **MINERAUX calculés/kg**		Nat. val.	CMV val.	TOTAL
P	mg	5 000	3 600	8 600
Ca	mg	6 000	4 600	10 600
Na	mg	1 500	1 950	3 450
K	mg	12 000	-	12 000
Mg	mg	3 000	130	3 130
Mn	mg	60	40	100
Fe	mg	170	150	320
Cu	mg	11	15	26
Zn	mg	40	45	85
Co	mg	0,1	1,5	1,6
I	mg	-	-	-
Cl	mg	-	-	-

VITAMINS calculated/kg **VITAMINES calculées/kg**		Nat. val.	CMV val.	TOTAL
Vitam. A	UI	4 000	15 000	19 000
Vitam. D3	UI	31	2 000	2 031
Vitam. B1	mg	6,5	16	22,5
Vitam. B2	mg	5	16	21
Vitam. B3	mg	23	100	123
Vitam. B6	mg	0,7	10	10,7
Vitam. B12	mg	0,004	0,05	0,054
Vitam. C	mg	-	800	800
Vitam. E	mg	15	250	265
Vitam. K3	mg	5	50	55
Vitam. PP	mg	93	100	193
Ac. Folic	mg	2,3	5	7,3
Ac. PAB	mg	-	10	10
Biotine	mg	0,025	0,25	0,275
Choline	mg	1 040	700	1 740
Meso-Inositol	mg	-	250	250

FICHE QUALITE 114

	Moyenne	Déviation standard	Limites de conformité
Quantité moyenne par lot (en tonnes)	10	± 3	
Contrôle de la composition centésimale (en %)	100,01	± 0,05	(99,5 à 100,5)

TECHNOLOGIE DES PELLETS

Diamètre (en mm)	3,17	± 0,04	(3,0 à 3,6)
Résistance à l'écrasement (en Kgf/cm²)	6,2	± 1,0	(3,0 à 11,0)
Résistance à l'abrasion (en %)	99,1	± 0,4	(>96)
Masse Spécifique (en g/l)	668,0	± 33,6	
Poids (en g)	0,077	± 0,010	
Longueur (en mm)	8,49	± 0,81	
Longueur < Diamètre (en %)	0,4	± 0,6	(<3)
Nombre de pellets chauffés par kg (/kg)	0	± 0	(<1)

CONTROLE DE LA QUALITE NUTRITIVE

Témoin incorporation mélange minéral (Na)	positif		
Témoin incorporation pré-mélange oligo-éléments (Mn et Cu)	positif		
Témoin incorporation pré-mélange vitamines (Vit A et C)	positif		
Eau (en %)	10,4	± 0,7	(8 à 13)
Protéines (en %)	19,9	± 0,6	(17 à 23)
Lipides (en %)	4,1	± 0,3	(3 à 5)
Glucides E.N.A. (en %)	44,9	± 0,6	(43 à 48)
dont Amidon (en %)	22,6	± 1,8	(16 à 28)
* Sucres totaux (en %)	3,0	± 0,8	
Cellulose WEENDE (en %)	13,5	± 0,5	(11 à 16)
Hémicellulose (en %)	10,7	± 2,0	
Cellulose vraie (en %)	13,3	± 1,2	
Lignine (en %)	3,0	± 0,1	
Minéraux totaux (en %)	7,2	± 0,3	(6 à 8,5)
dont Calcium (en mg/kg)	10800	± 600	(8000 à 13000)
* Phosphore (en mg/kg)	6300	± 300	(5000 à 8000)
* Sodium (en mg/kg)	2400	± 200	(1500 à 3000)
* Potassium (en mg/kg)	14500	± 1000	(10000 à 17000)
* Manganèse (en mg/kg)	85	± 9	(60 à 130)
* Cuivre (en mg/kg)	19	± 2	(≤35)
* Vitamine A (en UI/kg)	13100	± 1100	(7000 à 18000)
* Vitamine C (en mg/kg)	760	± 110	(400 à 1500)
* Vitamine D3 (en UI/kg)	1400	± 600	(≤3000)
* Vitamine E (en mg/kg)	190	± 50	

CONTROLE DES CONTAMINANTS

BACTERIOLOGIQUES				
Germes revivifiables (/g)	36400	± 45100	(<100000)
Moisissures & levures (/g)		<10	(<1000)
Coliformes totaux (/g)		0	(<5)
Coliformes fécaux (/g)		0	(0)
Anaérobies S.R. (/g)	18	± 22	(<100)
Salmonelles (/25 g)		0	(0)

METAUX LOURDS				
Plomb (en µg/kg)	360	± 200	(<1500)
Mercure (en µg/kg)	42	± 15	(<200)
Arsenic (en µg/kg)	150	± 80	(<1000)
Cadmium (en µg/kg)	81	± 43	(<250)
Sélénium (en µg/kg)	90	± 40	(<600)

MYCOTOXIQUES (en µg/kg)		
Aflatoxines	<1	(<5)
Ochratoxines	<12	(<200)
Zéaralénone	<50	(<1000)
Stérigmatocystine	<30	(<300)
Patuline		
Toxine T2		

DERIVES NITROSES			
NO2 (en mg/kg)	2	± 2	(Σ<500)
NO3 (en mg/kg)	280	± 10	
NDMA (en µg/kg)	0,96	± 1,02	(<10)
NDEA (en µg/kg)	<0,1		(<10)
NDPA (en µg/kg)	<0,15		(<10)
NDBA (en µg/kg)	<0,15		(<10)
NPIP (en µg/kg)	0,20	± 0,28	(<10)
NPYR (en µg/kg)	2,43	± 1,46	(<10)
NMOR (en µg/kg)	<0,3		(<10)

PESTICIDES ORGANOS-CHLORES
(en µg/kg) (Total <200)

Lindane	4	± 1	(<100)
a HCH	<1		(<100)
b HCH	<5		(<100)
d HCH	<5		(<100)
HCB	<1		(<50)
PCB	<50		(<50)
Aldrine	<1		(<10)
Dieldrine	<1		(<20)
Endosulfan	<1		(<100)
Heptachlore	<1		(<10)
Heptachlore Epoxyde	<1		(<10)
Endrine	<1		(<10)
o,p'DDD	<5		(Σ<50)
p,p'DDD	<5		
o,p'DDE	<1		
p,p'DDE	<1		
o,p'DDT	<5		
p,p'DDT	<5		

PESTICIDES ORGANOS-PHOSPHORES
(en µg/kg) (Total <7000)

Acéphate	<45	(<5000)	Iodofenphos	<25	(<5000)
Azinphos éthyl	<50	(<5000)	Malathion	38	± 27	(<5000)
Azinphos méthyl	<50	(<5000)	Méthamidophos	<10	(<5000)
Bromophos éthyl	<10	(<5000)	Méthidathion	<20	(<5000)
Bromophos méthyl	<20	(<5000)	Mévinphos	<10	(<5000)
Carbophénothion éthyl	<50	(<5000)	Monocrotophos	<90	(<5000)
Carbophénothion méthyl	<20	(<5000)	Naled	<15	(<5000)
Chlorfenvinphos	<25	(<5000)	Ométhoate	<20	(<5000)
Chlorméphos	<10	(<5000)	Oxydéméton méthyl	<400	(<5000)
Chlorpyriphos éthyl	<15	(<5000)	Parathion éthyl	<20	(<5000)
Chlorpyriphos méthyl	<15	(<1500)	Parathion méthyl	<20	(<5000)
Chlorthiofos	<100	(<5000)	Phosalone	<20	(<5000)
Diazinon	<15	(<5000)	Phosmet	<20	(<5000)
Dichlofenthion	<10	(<5000)	Phosphamidon	<25	(<5000)
Dichlorvos	<22	(<5000)	Profénofos	<20	(<5000)
Diéthion	<15	(<5000)	Prothoate	<20	(<5000)
Diméfox	<10	(<5000)	Pyridaphenthion	<15	(<5000)
Diméthoate	<30	(<1000)	Pyrimiphos éthyl	<20	(<5000)
Dioxathion	<15	(<5000)	Pyrimiphos méthyl	43	± 19	(<2500)
Disulfoton	<30	(<5000)	Sulfotep	<20	(<5000)
Ethoprophos	<20	(<5000)	Témephos	<15	(<5000)
Fenchlorphos	<20	(<5000)	Tétrachlorvinphos	<30	(<5000)
Fénitrothion	<15	(<5000)	Thiméthon	<40	(<5000)
Fenthion	<30	(<5000)	Triazophos	<30	(<5000)
Fonofos	<20	(<5000)	Trichlorfon	<10	(<5000)
Formothion	<15	(<5000)	Trichloronate	<25	(<5000)
Hepténophos	<30	(<5000)			

1995

Phycher

Bio développement

STUDY ORGANISATION

Assessment of sensitising properties on albino guinea pigs
Maximisation test according to MAGNUSSON and KLIGMAN

References : *Guideline O.E.C.D. N° 406 of July 17th 1992*
Directive B.6-96/54/EEC of July 30th 1996 (E.E.C. Journal L248)
Magnusson B and Kligman A.M., (1969) – The identification of contact allergens by animal assay. The guinea pig maximisation test. J. Invest. Dermatol. , 268-276.

Test will be performed under the experimental protocol SMK - version February 2002.

Test product : Mannostab[MD] **Batch** : 10064/06-2000

Sponsor :
Laffort Oenologie
Quai de Souys
BP 17
33015 BORDEAUX Cedex

Study monitor:
ADEC TOX
[redacted]
120, rue quintin
33000 BORDEAUX

Testing facility : PHYCHER *Bio Développement*
18 chemin de Lou Tribail
ZA de Toctoucau
33610 CESTAS

Study director : [redacted]

Schedule study:

Start of study :	Week 09*	
End of study:	Week 14	Results by fax
Draft	Week 16	
Final report:	Week 17	2 reports (english)

* If signing of the protocol by the study monitor week 08

Approbation

Date : [redacted]

Study [redacted]

Date : [redacted]

Study Monit [redacted]

EXPERIMENTAL PROTOCOL
REF. SMK

Version February 2002

Assessment of sensitising properties on albino guinea pigs
Maximisation test according to MAGNUSSON and KLIGMAN

References :

- ***Guideline O.E.C.D. N° 406 of July 17th, 1992***
- ***Directive B.6 96/54/E.E.C. of July 30th, 1996 (E.E.C. Journal L248 dated September 30th, 1996)***
- ***Magnusson B and Kligman A.M., (1969) – The identification of contact allergens by animal assay. The guinea pig maximisation test. J. Invest. Dermatol. 52, 268-276.***

1 - INTRODUCTION

This protocol is established according to :

- the O.E.C.D. Guideline N° 406 dated July 17th, 1992 and

- the Directive B.6 96/54/E.E.C. dated July 30th, 1996 (E.E.C. journal L248 dated September 30th, 1996) relative to research on sensitisation of the skin.

The method retained is an adaptation of the technique described by Magnusson and Kligman.

It is an early detection method, and the results obtained provide an estimate of the cutaneous sensitising potential of the test product. Extrapolation to humans remains limited, especially with regards to products revealing low sensitising potential on animals.

Before any test, an experiment overview dated and signed by the study director is established and submitted to the management in order to define the object of the study and its development.

These documents are applied and used in compliance with the Principles of the Good Laboratory Practices (G.L.P.).

2 - PRINCIPLE

Guinea pigs are firstly submitted to the test product (induction phase) by intradermal injection, or topical application. After at least a 14-day rest phase (induction period) during which an immune response can develop a single application of that same product is performed in order to discover whether a state of hypersensitivity is induced (challenge phase).

3 - ANIMALS USED AND HOUSING CONDITIONS

3.1 - Animals

Albino guinea-pigs Dunkin-Hartley strain, weighing between 300 and 400 g (± 50 g) at the beginning of the test. Each group must be homogeneous in weight.

3.2 - Housing

The animals are housed either in groups of 2 or 3 in makrolon containers, dimensions 47 cm x 31 cm x 19 cm, the flooring of which is covered with dust-free cuttings (UAR 91360 Villemoisson s/Orge) and the top fitted a stainless steel lid with a feeding device and drinking device of 500 ml.

The containers are placed in an air-conditioned animal holding facility :

- Air recycling: at least 10 cycles per hour,
- Temperature: 22° C ± 3° C,
- Relative humidity: from 30 % to 70 %,

which circadian cycle : 12 hrs day / 12 hrs darkness.

The extreme values for the temperature and the relative humidity during the test are included in the final report.

3.3 - Food and drink

The drinking water (tap water from public distribution system) and food (UAR 114 - UAR 91360 Villemoisson s/Orge) are supplied freely.
Microbiological verification and chemical analysis are conducted every six months by the Institut Européen de l'Environnement de Bordeaux (I.E.E.B.).

4 - OPERATING METHOD

4.1 - Preparation of animals

Prior to the test, the animals are kept for a minimum acclimatisation period of 5 days, under stabling and nutritional conditions identical to those of the test.

Before the experimentation process, they are identified individually by marking with picric acid and a tattoo placed on their ear.

The animals are carefully shorn before each product application :
- on the inter-scapular zone for the induction phase.
- on the dorso-lumbar zone for the challenge phase.
At least 3 hours before the first reading (challenge phase) they are shorn a second time in this dorso-lumbar zone.

The animals are weighed at the beginning and at the end of the study.

4.2 - Characteristics of the test product

The test product is to be coded upon reception by the laboratory, using the operating procedure. The characteristics of the product (conditioning, quantity, form, colour...) are to be logged in the data base and completed, if necessary, upon opening the sample in the laboratory.

The test product is subsequently stored in a suitable place in the laboratory at room temperature or 4°C in the fridge in accordance with information applied by the Sponsor.

All available information (safety data sheet) on the test product should be made available to the laboratory. Information concerning the identity, purity and the stability of the test product are the responsibility of the Sponsor. An analysis form or certificate for the sample under investigation was requested from the Sponsor who ordered the test and enclosed with the final report.

Notes:

- *the solid products are :*

-dissolved or put into suspension in an appropriate vehicle (solution of sodium chloride for intradermic injection, distilled water for topical application, oil ...).
-or applied as they are.

- *the liquid or somewhat thick products are applied as they are or diluted in an appropriate vehicle.*

4.3 - Preliminary tests

4.3.1 - Determination by intradermal injection of the Maximal Non Necrotizing Concentration (MNNC)

This test is conducted for the purpose of defining a MNNC which, on injection during the induction phase, does not risk causing too great a lesion (non-necrotizing concentration).

Two animals receive on both sides of the spine, a volume of 0.1 ml of the test product, at the following concentrations : pure (100%), 50%, 25%, 12.5%, 6.25%, 3.125%. Taking into account these concentrations are necrotizing, an another group of 2 animals is used to test lower concentrations.

A macroscopic evaluation of the cutaneous reactions is conducted 24 hours later and 48 hours later if necessary.

4.3.2 - Determination by topical application of the Maximal Non Irritant Concentration (MNIC)

4.3.2.1 - For the induction phase (Pre-MNIC)

This test, which allows to evaluate the irritant potential of the test product, will define whether an application of sodium lauryl sulfate will be made during topical induction phase.
The test product is applied on the dorso-lumbar zone of two guinea pigs shorn beforehand, with occlusive dressing for 24 hours, at different concentrations (pure (100%), 50%, 25%, 12.5%).

A macroscopic evaluation of the cutaneous reactions is conducted 24 hours after removal of the dressing.

4.3.2.2 - For the challenge phase (MNIC)

This test is carried out for the purpose of determining the MNIC without risk of an irritant effect during the challenge phase.

Three guinea pigs are treated according to the same treatment as animals from GROUP 1 (negative control) for the induction phase (paragraph 4.4.2), main study.
During the challenge phase, the animals are treated with the test product placed onto the selected treatment sites and covered with an occlusive dressing for a period of 24 hours at 4 different concentrations determined from the Pre-MNIC results.

A macroscopic evaluation of the cutaneous reactions is conducted 24 hours after removal of the occlusive dressing.

4.4 - Main study

4.4.1 - Allocation of groups

This study is performed with two groups of animals:

- **GROUP 1 (Negative control)** : at least 10 guinea pigs used for the verification during the challenge phase of the absence of any primary cutaneous irritation effect.
- **GROUP 2 (Treated)** : at least 20 guinea pigs used for the assessment of any possible sensitisation property of the test product.

A third group treated with a reference allergen may be selected, at the request of the Sponsor.

Alternatively, the sensibility of the method is checked at least once a year using substances which are known to have mild to moderate skin sensitisation properties (benzocaine and neomycin sulfate).

NOTE
In accordance with the O.E.C.D. Guideline n°406, in a first step 5 animals were retained for the control group and 10 for the treated one. In these conditions, if results enable an interpretation, the test can be validated. On the contrary, it must be completed by another test consisting in 5 controls and 10 treated animals.

4.4.2 - Induction phase

4.4.2.1 - For the products allowing intradermal injection.

1ST Intradermal Induction :

Day 0

After shearing the scapular zone, three (3) pairs of intradermal injections (II) of 0.1 ml are performed on the scapular zone in such a way as an injection on each pair is placed to either side of the spine as follows :

GROUP 1 (Negative control):
- 2 ID : Freund's Complete Adjuvant diluted at 50 % in an injectable isotonic solution of sodium chloride (NaCl).
- 2 ID - an injectable isotonic solution of NaCl
  - or appropriate vehicle in the case of product preparation.
- 2 ID a mixture with equal volumes v/v :
  - Freund's Complete Adjuvant at 50% and NaCl,
  - or Freund's Complete Adjuvant at 50% and appropriate vehicle.

GROUP 2 (Treated):
- 2 ID : Freund's Complete Adjuvant diluted by 50 % in an injectable isotonic solution of NaCl,
- 2 ID : the test product at a concentration slightly irritant if possible (MNNC),
- 2 ID a test mixture in equal volumes v/v :
  - Freund's Complete Adjuvant at 50% and the test product at the final concentration slightly irritant.

2nd Topical induction:

Day 6 ± **1** (only if the test product is not intrinsically irritant)

The scapular zone of all the animals in each group, shorn beforehand, is brushed with a solution of sodium lauryl sulfate at 10% in thick vaseline, in order to create a local irritation.

Day 7 ± **1**

A topical application under occlusive dressing for 48 hours is performed on the injection sites of each animal.

GROUP 1 (Negative control) : 0.5 ml of isotonic solution of sodium chloride, vehicle or solvent alone.

GROUP 2 (treated) : 0.5 ml (or g) of the pure test product.

4.4.2.2 - **For the products not allowing by intradermal injection**

1st Topical induction :

Day 0 :

After shearing of the scapulary zone, it is carried out :

GROUP 1 (Negative control):

- 2 pairs of intradermal injections (ID), on either side of the spine, Freund's Complete Adjuvant diluted at 50 % in an injectable isotonic solution of NaCl, under a volume of 0.1 ml (ID),
- 3 scarifications performed between injection sites,
- an application of 0.5 ml (or g) of the vehicle or solvent during 48 hours under semi-occlusive dressing.

GROUP 2 (Treated):

- 2 pairs of intradermal injections (ID), on either side of the spine, Freund's Complete Adjuvant diluted at 50 % in an injectable isotonic solution of NaCl, under a volume of 0.1 ml,
- 3 scarifications performed between the injection sites,
- an application, on the scarified zone, of 0.5 ml (or g) of the test product, if possible, at a slightly irritant concentration during 48 hours under semi-occlusive dressing.

2nd Topical induction:

Day 6 ± **1** (only if the test product is not intrinsically irritating)

The scapular zone of all the animals of each group, shorn beforehand, is brushed with a solution of sodium sulfate lauryl at 10 % in thick vaseline in order to create a local irritation.

Day 7 ±1

A topical application under occlusive dressing, for 48 hours, is performed on the injection sites.

GROUP 1 (Negative control) : 0.5 ml of vehicle or solvent,

GROUP 2 (Treated) : 0.5 ml (or g) of the pure test product.

4.4.3 - **Rest phase**

The animals of both groups are left at rest for at least 14 days.

4.4.4 - **Challenge phase**

Day 28

The experimental procedure of this phase is identical for both groups **GROUP 1 (Negative control) and GROUP 2 (Treated)** submitted to this experimentation : on the previously shorn dorso-lumbar zone, an application on either side of the spine, under occlusive dressing (FINN CHAMBER sample cup-Promédica covered with hypoallergenic strip), is performed during 24 hours :

- 1 sample cup containing the test product at MNIC (concentration determined during preliminary test (cf. 4.4.2.2) and 1 sample cup containing the test product at 1/2 MNIC.

5 - MACROSCOPIC EXAMINATIONS AND EVALUATION OF CUTANEOUS REACTIONS

A macroscopic evaluation of the cutaneous reactions (erythema and oedema) is conducted and all the local or systemic reactions are recorded as **GROUP 1 (Negative control) and GROUP 2 (Treated) :**

- Approximately 21 hours after removal of the occlusive dressing, the treated zone is shorned, and if necessary cleaned,
- Approximately 3 hours later, the cutaneous reactions will be observed and graded according to the scales, given below.
- 24 hours later (i.e. 48 hours after removal of the occlusive dressing), a second observation will be made.

Grading scales :

Erythema	Oedema
0..... No visible modification	**0** .... No visible modification
1..... Slight or patches of erythema	**1** .... Slight odema
2..... Moderate confluent erythema	**2** .... Moderate oedema
3..... Internal erythema and swelling	**3** .... Important oedema

NOTE :

- *Insofar as possible, the observation of the animals treated and control will be conducted double blindly.*
- *All other reactions which could indicate an allergic reaction (pimple or vesicles) must be recorded and taken into consideration in the interpretation.*
- *Reading of the preliminary test will be carried out according to the same grading scale.*
- *In case of doubtful reactions, the animals will be held for re-challenge after a period of no less than one week, with the agreement of the sponsor. Moreover the determination of skin thickness fold may be carried out. A skin biopsy at each treated site may be carried out and preserved in an appropriate fixation liquid for histological examination.*
- *Readings may be conducted until 96 hours with regard the reactions.*

6 - INTERPRETATION OF REACTIONS

All the animals with scores (erythema or oedema) above or equal to 2 during the challenge phase, are considered positive.

The percentage of animals that showed a contact sensitivity potential are calculated from the reading at 24 and 48 hours.

7 - INTERPRETATION OF RESULTS

The experimental sensitising potential of the test product is established according to the following scale:

PERCENTAGE (%) OF SENSITISED ANIMALS	CATEGORY	CLASSIFICATION
0 - 8	I	VERY LOW
> 8 - 28	II	MILD
>28 – 64	III	MODERATE
>64 - 80	IV	STRONG
>80 - 100	V	EXTREME

In accordance with the E.E.C. Directives 93/21 (O.J.E.C.L110 A, May 4th , 1993), 91/325 dated March 5th , 1991 (O.J.E.C. L 180 dated July 8th , 1991) and 67/548, the results obtained for products can be classified according to European regulations concerning the classification, packaging and labelling of dangerous substances:
The substances or preparations will be classified as sensitising and characterised by the symbol "Xi" and the danger label *"irritant"* with the risk sentence R43 in accordance with the criteria below:

- **R 43 : may cause sensitisation by skin contact**

➢ If experience shows that substances or preparations can provoke a sensitisation reaction by skin contact in a significant number of people or if tests carried out in animals give positive results. In case of tests methods used for skin contact sensitisation according to Appendix V or, in case of other trials methods using an adjuvant, an answer over at least 30% of animals is regarded as positive.

8 - AMENDMENTS TO THE PROTOCOL

Any modification to the experimental conditions defined in this protocol, will be included in additional clauses, with the agreement of the Study Director and the Sponsor.

9 - QUALITY ASSURANCE

The study carried out in accordance with the protocol, the experiment overview and the generated transcript data are inspected by the Quality Assurance Unit of PHYCHER *Bio Développement*.

Inspection of the toxicity tests are performed and conducted according to a process for verification of the different major technical phases concerning at least one similar test. The frequency is one or more inspection per term.

The different inspections are carried out in compliance with the Good Laboratory Practices (G.L.P) established by the decisions concerning *the mutual acceptance of data in the evaluation of chemical substances, (C(81) 30 (final) appendix 2, May 12th, 1981; C (97) 186, November 26th, 1997)*, and transcribed in the *decree n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française.*

10 - FINAL REPORT

The final report will contains at least the following minimal information :

- Description of the test product and vehicle or solvent used (if necessary)
- Species, sex, strain of animals used as well as the number and supplier.
- Type of food used and acclimatisation and environmental conditions.
- Conditions under which the study was conducted.
- Results of all the individual observations presented as table.
- Description of the degree of cutaneous reactions and type.
- Percentage of the sensitised animals.
- Description of observations relative to unusual findings or reactions.
- Classification according to regulations with the risk phases and symbols.

11 - ARCHIVES

The protocol and amendments to the protocol, the original data, correspondence, and final report are kept in a location specially reserved for this purpose at PHYCHER *Bio Développement* for a 10-year period.

At the end of this period, the study archives will be either returned to the Sponsor of the study or destroyed.

The test product , from its initial container, is :
- sent back to the Sponsor, at his request, so no storage is done,
- generally kept in the original container 10 years after emission of the final report.

PROTOCOL APPROVED:

Date : 02 / 21 / 2002

[redacted]

Study Director
(Appointed at the start of the study)

MODIFICATION TO THE STANDARD EXPERIMENTAL PROTOCOL

The following modifications will be applied to the standard experimental protocol:

ACCORDING TO THE G.L.P.
DESCRIBED IN 88/320 E.E.C. AND
C/81/130 O.E.C.D. DIRECTIVES
BY G.I.P.C. 10/23/98

Phycher
Bio développement

Assessment of acute irritant/corrosive effect on the skin

Final Report : IC-OCDE-PH-02/0051

TEST PRODUCT
Mannostab^{MD}
Batch n°10064/06-2000

❑ **STUDY MONITOR** : **ADEC TOX**
[redacted]
120, rue Quintin
F-33000 BORDEAUX

❑ **SPONSOR** : **LAFFORT OENOLOGIE**
Quai de Souys
BP 17
F-33015 BORDEAUX Cedex

Report of 9 pages and 0 appendix **Cestas, April 02nd, 2002**

CONTENTS

QUALITY ASSURANCE ATTESTATION

I, the undersigned Bernard Benech, Quality Assurance Unit of PHYCHER *Bio développement*, attest that the study **IC-OCDE-PH-02/0051** was submitted to the inspection of Quality Assurance.

The routine inspections of the toxicity tests performed within the laboratory of PHYCHER *Bio développement*, are carried out as a control process of different major technical phases concerning at least one similar test. The frequency is once a term or more. Dates of technical phases inspections relating to this test are given below :

Date of inspection	: February 26th, 2002 *Test product application at D0*
Date of reporting to Study Director	: February 26th, 2002
Date of reporting to Management	: February 26th, 2002

This report has been audited by PHYCHER *Bio développement* Quality Assurance unit. It is considered to be an accurate account of the data generated and the application of the operating procedures in use within the laboratory:

	Draft report	Final report
Date of audit report	: March 14th, 2002	April 02nd, 2002
Date of reporting to Study Director	: March 14th, 2002	April 02nd, 2002
Date of reporting to Management	: March 14th, 2002	April 02nd, 2002

Date April 02nd, 2002

[redacted]

AUTHENTICATION

I, the undersigned, François Richeux, Study director, certify that the study **IC-OCDE-PH-02/0051** was performed on the premises of the Laboratory PHYCHER *Bio développement* under the technical responsibility of Corinne Scheyder.

I, certify that the objectives laid down in the technical protocol were achieved and no undesirable event occurred to affect the quality or the integrity of the study. I consider the data generated to be valid. This report fully and accurately reflects the operating procedures used and data generated.

The entire work was performed in compliance with the principles of the Good Laboratory Practices (G.L.P.), as defined in *the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) Appendix 2 - May 12th, 1981; C (97) 186, November 26th, 1997),* and transcribed in the *decree n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française.*

Date Avril 02 nd 2002

[redacted]

Study Director

SUMMARY AND CONCLUSION OF THE STUDY

The product **Mannostab**[MD] was applied, as supplied, at the dose of 0.5g, under semi-occlusive dressing during 4 hours on an undamaged skin area of 3 rabbits, according to an experimental protocol established from *the O.E.C.D. guideline (n° 404 dated July.17th,1992) and the method B.4 of the E.E.C. directive n° 92/69 dated December 29th,1992.*

No cutaneous reactions (erythema and oedema) were observed in any animal whatever the examination time.

The results obtained, in these experimental conditions, enabled to conclude that the test product **Mannostab**[MD], according to the scales of interpretation retained :

- is **non irritant** to skin (PSi = **0**) according to the classification established in *the Journal Officiel de la République Française dated February 21st, 1982,*

- and, **must not be classified,** according to the criteria for classification, packaging and labelling of dangerous substances in compliance with *the E.E.C. Directives 67/548 and 93/21.*

Date Avril 02 nd 2002

Scientific Direction PHYCHER *Bio développement*

[redacted]

Pharmacologist
(Hdr)

cofrac ESSAIS
ACCREDITATION N° 1-0887

TEST REPORT

1 - TEST PRODUCT

The test product **Mannostab**^{MD}, sent by ADEC TOX - 120 rue Quintin – F-33000 Bordeaux FRANCE, was received on 02/20/02. Its characteristics were:

- Container : plastic pilular (n=3)
- Quantity : 80.97g (container + contents)
- Batch : 10064/06-2000
- Form : powder
- Colour : whitish
- Storage : room temperature

It was identified under the code number: **PH-02/0051**.

Informations concerning the identity, purity and stability of the test product are the responsibility of the Sponsor. An information data sheet concerning the product was provided by the study monitor.

2 - TEST DEVELOPMENT

The study was carried out between 02/26/2002 and 03/01/2002, in *PHYCHER Bio développement - 18 chemin de Lou Tribail - ZI de Toctoucau - F-33611 Cestas Cedex-* according to *the protocol Ref. IC-O.C.D.E. - version September 1999,* established according to *the O.E.C.D. n° 404 dated July 17th, 1992 guideline concerning dermal acute irritation/corrosion and the method B.4 of the E.E.C. directive n° 92/69 dated December 29th, 1992.*

2.1 - Animals

3 male albinos rabbits, numerated A4509, A4510 and A4511 of new-zealand strain, originating from the Elevage de Gérome (Quartier Labaste – 40260 Linxe) were kept during a 14-day acclimatisation period. During the test, the animals weighted between 2.29kg and 2.59kg.

The animals were kept in individual boxes installed in conventional air conditioned animal husbanding; the environmental conditions were:

- temperature : between 20 °C and 22 °C
- relative humidity : between 45% and 52%

2.2 – Treatment

The test product was applied, as supplied, at a dose of 0.5g, on an undamaged skin area of the right flank of each animal.
On the left flank was applied, in the same experimental conditions, a dose of 0.5mL of distilled water on an undamaged skin area.

2.3 – Grading of reactions

The skin reactions were appreciated 1 hour and then 24, 48 and 72 hours after removal of the patch.

NOTE:

If no reaction is observed at 72 hours (D3), the test is completed. In case of persistent reactions, additional observations can be carried out from Day 4 (D4) to Day 14 (D14) if necessary in order to determine the reversible character of the lesions observed.

3 - RESULTS

The individual and mean scores obtained during the study are shown in *tables 1 to 3* hereafter.

No cutaneous reactions (erythema and oedema) were observed in any animal whatever the examination time.

4 - CONCLUSION

The results obtained, in these experimental conditions, enabled to conclude that the test product **Mannostab**[MD], according to the scales of interpretation retained:

- is **non irritant** to skin (PSi =**0**) according to the classification established in *the Journal Officiel de la République Française dated February 21st, 1982,*

- and, **must not be classified,** according to the criteria for classification, packaging and labelling of dangerous substances in compliance with *the E.E.C. Directives 67/548 and 93/21.*

5 - ARCHIVES

All the original data and the final report related to this study will be stored for a period of 10 years in the company under the reference **IC-OCDE-PH-02/0051**. After this period, the Sponsor's instructions will be applied.

6 - PROTOCOL ADHERENCE

No deviation was registered during this study.

Date 03.01.2002

Technical Responsible

[redacted]

ASSESSMENT OF THE ACUTE IRRITANT/CORROSIVE EFFECT ON THE SKIN

TEST PRODUCT : Mannostab^{MD}

Application : 0.5g of pure test product

Operator : [redacted]

Application date : 02/26/2002

Number of animals : 3

Table 1

INDIVIDUAL AND AVERAGE SCORES AFTER 4-HOUR EXPOSITION

(In compliance with the E.E.C. regulation)

OBSERVATIONS		INDIVIDUAL DATA						TOTAL OF INDIVIDUAL DATA
Skin reactions	Observation time	Animal n°	Weight (kg)	Animal n°	Weight (kg)	Animal n°	Weight (kg)	
		A4509	Start: 2.53 End: 2.59	A4510	Start: 2.49 End: 2.55	A4511	Start: 2.29 End: 2.39	
Erythema and eschar	24 hours	0		0		0		0
	48 hours	0		0		0		0
	72 hours	0		0		0		0
Total		0		0		0		
Mean		0.0		0.0		0.0		
Oedema	24 hours	0		0		0		0
	48 hours	0		0		0		0
	72 hours	0		0		0		0
Total		0		0		0		
Mean		0.0		0.0		0.0		
CLASSIFICATION		According to the calculated means						
		the product must not be classified in accordance with the European regulation						

ASSESSMENT OF THE ACUTE IRRITANT/CORROSIVE EFFECT ON THE SKIN

TEST PRODUCT : Mannostab[MD]

Application : 0.5g of pure test product

Operator : [redacted]

Application date: 26/02/2002

Number of animals: 3

Table 2

DETERMINATION of SKIN IRRITATION INDICES

(In compliance with the Journal Officiel de la République Française)

OBSERVATIONS		INDIVIDUAL DATA						TOTAL OF INDIVIDUAL DATA
Skin reactions	Observation time	Animal n°	Weight (kg)	Animal n°	Weight (kg)	Animal n°	Weight (kg)	
		A4509	Start: 2.53 End: 2.59	A4510	Start: 2.49 End: 2.55	A4511	Start: 2.29 End: 2.39	
Erythema and eschar	24 hours	0		0		0		0
	72 hours	0		0		0		0
Oedema	24 hours	0		0		0		0
	72 hours	0		0		0		0
RESULTS		TOTAL SUM OF REGISTERED SCORES						0
		PRIMARY SKIN IRRITATION : PSi						0.0
Classification according to the Journal Officiel de la République Française dated February 21st, 1982								*Non irritant*

ASSESSMENT OF THE ACUTE IRRITANT/CORROSIVE EFFECT ON THE SKIN

TEST PRODUCT : Mannostab[MD]

Application : 0.5g of pure test product

Operator : [redacted]

Application date: 02/26/2002

Number of animals: 3

Table 3

INDIVIDUAL SKIN REACTIONS

OBSERVATIONS		INDIVIDUAL DATA						TOTAL OF INDIVIDUAL DATA
Skin reactions	Observation time	Animal n°	Weight (kg)	Animal n°	Weight (kg)	Animal n°	Weight (kg)	
		A4509	Start: 2.53 End: 2.59	A4510	Start: 2,49 End: 2.55	A4511	Start: 2.29 End: 2.39	
Erythema and eschar	1 hour	0		0		0		0
	24 hours	0		0		0		0
	48 hours	0		0		0		0
	72 hours	0		0		0		0
Oedema	1 hour	0		0		0		0
	24 hours	0		0		0		0
	48 hours	0		0		0		0
	72 hours	0		0		0		0

Phycher

Bio développement

STUDY ORGANISATION

ASSESSMENT OF ACUTE IRRITANT/CORROSIVE EFFECT ON THE SKIN

References : *O.E.C.D. Guideline n_o 404 dated July 17th, 1992*
E.E.C. Directive n_o 92/69 dated July 31st, 1992 (E.E.C. Journal L383 A dated December 29th 1992)

Test will be performed under the experimental protocol IC-OCDE - version September 1999

Test product : Mannostab[MD] **Batch** : 10064/06-2000

Sponsor :
Laffort Oenologie
Quai de Souys
BP 17
33015 BORDEAUX Cedex

Study monitor:
ADEC TOX
[redacted]
120, rue quintin
33000 BORDEAUX

Testing facility :: PHYCHER *Bio développement*
18 Chemin de Lou Tribail
ZA de Toctoucau
33610 CESTAS

Study director : [redacted]

Schedule study:

Start of study :	Week 09*	
End of study:	Week 10	Results by fax
DRAFT :	Week 12	-
Final report:	Week 13	2 reports (english)

* If signing of the study organisation by the study monitor week 08

Approbation

Date : Feby 21th 2002
[redacted]

Date : 1.3.02
Study [redacted]

EXPERIMENTAL PROTOCOL
REF: IC-O.C.D.E.

Version september 1999

ASSESSMENT OF ACUTE IRRITANT/CORROSIVE EFFECT ON THE SKIN

References:

- ***O.E.C.D. Guideline n_o 404 dated July 17th, 1992***
- ***E.E.C. Directive n_o 92/69 dated July 31st, 1992 (E.E.C. Journal L383 A dated December 29th, 1992)***

1 – INTRODUCTION

This protocol has been devised in accordance with :

- *O.E.C.D. Guideline n_o 404 dated July 17th, 1992,*

- *E.E.C. Directive n_o 92/69 dated July 31st, 1992 (E.E.C. Journal L383 A dated December 29th, 1992), relating to the evaluation of acute irritant/corrosive effects of chemical substances on the skin.*

Before any test, an experiment overview dated and signed by the study director is established and submitted to the management in order to define the object of the study and its development.

These documents are applied and used in compliance with the Principles of the Good Laboratory Practices (G.L.P.).

2 – PRINCIPLE

The test product is to be applied to the skin of three rabbits in a single dose. Each rabbit is its own control. The degree of irritation is assessed at set intervals.
The test is conducted over a sufficient length of time, at least six days, in order to determine the reversibility or irreversibility of the effects observed. There is generally no need to exceed 14 days post-application.

All available information concerning the product to be tested should be taken into consideration in order to limit tests liable to produce serious reactions, such as :

- ***Acidic products of pH 2.0 or less, and alkaline products of pH 11.5 or greater with corrosive properties;***
- ***Products for which in vitro tests suggest the likelihood of irritant, severely irritant or corrosive properties. In this case, a reduced test will be performed after an advice of the toxicologist expert.***

NOTE:

In the cases mentioned above, the test may not need to be performed, a single animal will be used to confirm the predicted serious effects. In the total absence of information, the test is to begin with a single animal.

3 – ANIMAL USED AND HOUSING CONDITIONS

3.1 – Animals

Three albino New Zealand strain rabbits of the same sex, male or female, aged approximately 11 weeks, and generally weighing between 2.2 and 2.5 kg at the start of the test, are to be used.
Where results are not conclusive, a larger number of animals may be required, after approval by the Sponsor.

3.2 - Housing

The animals are housed in individual wire floored stainless steel cages of dimensions 61 cm x 46 cm x 34 cm. The door of each cage is fitted with a feeding dish and a drinking device of 1.000ml.

The cages are placed in an air-conditioned animal holding facility :

- Air recycling: at least 10 cycles per hour,
- Temperature: 20° C ± 3° C,
- Relative humidity: from 30 % to 70 %,

which circadian cycle : 12 hrs day / 12 hrs darkness.

The extreme values for the temperature and the relative humidity during the test are included in the final report.

3.3 – Food and drink

Drinking water (tap-water from public distribution system) and foodstuff (UAR 112 from UAR in 91360 Villemoisson s/Orge, France) are supplied freely.
Microbiological and chemical analyses of the water are carried out once every six months by the Institut Européen de l'Environnement de Bordeaux (I.E.E.B.).

4 - OPERATING PROCEDURE

4.1 – Preparation of the animals

Prior to the test, the animals are to be kept during an acclimatisation period of at least 5 days, under the same living and feeding conditions as for the test.

They are to be identified on the day of arrival by a numbered metal ring (Chevillot-Quick) attached to the side of the ear.

Approximately 24 hours before the product is applied, the rabbits' backs and flanks are shorn using electric clippers equipped with a fine comb, so as to expose an area of skin about 6 cm^2.

Any animal with skin lesions is replaced.

4.2 – Characteristics of the test product

The test product is to be coded upon reception by the laboratory, using the operating procedure. The characteristics of the substance (conditioning, quality, form, colour...) are to be logged within the data base form and completed, if necessary, upon opening the sample in the laboratory.

The test product is subsequently stored in a suitable place in the laboratory at room temperature or 4°C in the fridge in accordance with information applied by the Sponsor.

All available information (safety data sheet) on the product under investigation should be made available to the laboratory. Information concerning the identity, purity and the stability of the test product are the responsibility of the Sponsor. An analysis report or certificate for the sample under investigation is requested from the Sponsor who ordered the test and enclosed with the final report.

4.3 – Treatment

The test product is applied onto the shorn skin (unaffected zone) on the right side, the left side is covered in the same conditions with distilled water (generally used reference excipient). Each area is then covered with a square hydrophilic Codex of 8-layer absorbent gauze, approximately 2.5 cm square, which is subsequently maintained in position on the skin by a semi-occlusive dressing. The dressing is covered with an elastic strap taped in place around the animal's body, taking care not to impede its respiratory an abdominal movements.

The product is kept four hours in contact with the skin, with dosage being :

- *for solid or powdery products : 0.5g moistened beforehand with distilled water or other appropriate excipient to ensure a good contact with the skin. Solid products may be powered if required,*
- *for liquid and pasty products : 0.5ml or 0.5g placed directly on the skin.*

Depending on the conditions of use of the test product, and/or its texture, a modified preparation may prove necessary. In such cases, the influence of the excipient is taken into account, and this latest will be studied alone on the opposite side.

NOTES :

- *where the product is suspected of producing a sever irritant or corrosive effect an initial test is to be planned with a single animal in the same experimental conditions described above. It must be consider to apply 3 compresses bearing the product simultaneously to the same animal. The first is removed after three minutes. If no severe skin lesions are apparent, the second compress is removed after one hour. If the reactions observed at this point indicate that the animal can be left exposed to the substance for four hours without suffering, the third compress is to be removed after four hours. After each time, the skin reactions are graded.*

- *at the outcome of the application period, any residual product is removed, if necessary using a swab soaked in distilled water or a suitable solvent, taking care not to affect the skin.*

- *longer product-skin contact times may appear necessary in certain circumstances, depending for example on the anticipated use an exposure times in humans.*

5 – MACROSCOPIC EXAMINATION AND EVALUATION OF SKIN IRRITATION

One hour then 24, 48 an 72 hours after removal of the semi-occlusive dressing, the animals are to undergo a macroscopic skin examination, on each occasion under the same conditions (in particular with regard to ambient lighting).

This examination consists in assessing the irritant reactions in the treated zone, compared to a control area ; the following scales are used :

ERYTHEMA AND ESCHAR FORMATION

- No erythema **0**
- Slight (barely perceptible) erythema **1**
- Definite erythema **2**
- Moderate to severe erythema **3**
- Severe erythema (purple) with formation of eschars (deep lesions) preventing erythema from being graded **4**

NOTE :

- *where erythema cannot be graded due to significant coloring by the product tested, other means of assessment, such as cell examination may be implemented, with the approval of the Sponsor of the study.*

ŒDEMA

- No œdema **0**
- Very slight (barely perceptible) œdema **1**
- Slight oedema (contour clearly defined by a marked swelling) **2**
- Moderate oedema (thickness approx. 1 mm) **3**
- Severe oedema (thickness greater than 1 mm, an surface larger than zone of application) **4**

NOTES :

- *If no signs of irritation persist 72 hours after removal of the dressing, the test is to be conclude.*
- *Where skin lesions remain, the observation period may be extended from the 4th to 14th day, in order to determine the reversibility or irreversibility of the lesions.*
- *Any modifications in skin structure, mainly dryness, roughness, loss of litheness, scab are to be noted according to the following numeration :*

1 : slight dryness
2 : dryness
- *3 : roughness/loss of litheness*
- *4 : outbreak of scab*

- *Where the choice between two values is unclear, the higher value is to be chosen.*

6 – EXAMINATION OF THE GENERAL CONDITION OF THE ANIMALS

The systemic toxicity, expressing by behavioural abnormalities, alteration of neuro-vegetative reactions or decrease feeding, and any toxic effects are to be logged and described.
The animals are weighed at the start and at the end of the test.

7 – INTERPRETATION OF THE RESULTS

7.1 – Interpretation according to European regulations

For the products, the mean score obtained can be applied according to the E.C.C. Regulation of the dangerous substances. In accordance with *E.E.C. Directives 93/21 (E.E.C. Journal L110A dated May 4th, 1993), 91/325 dated March 5th, 1991 (EEC Journal L180 dated July 8th, 1991), and 67/548,* relating to the labelling, packaging and classification of dangerous substances, mean values are to be calculated for observations made at 24, 48 and 72 hours. This mean enables products to be classified as follows:

- **Corrosive substance or preparation**

An substance or preparation is classified corrosive if, when applied to healthy and unbroken animal skin, it causes tissue destruction in all skin layers, in at least one animal, during skin irritation tests carried out in accordance with *E.E.C. Directive 92/69 or equivalent method*, or where results are predictable (e.g. strongly acidic [pH ≤ 2] or alkaline [pH ≥ 11.5] substances).

The substance is to be classified as corrosive and characterised by the symbol "C" and the warning label "corrosive".

The corresponding hazard warning is :

- **R35 : causes severe burns**

➢ If the substance or preparation applied to the unbroken healthy skin of an animal causes tissue destruction in all skin layers after an exposure of three minutes or less, or where such a result may be predicted.

- **R34 : causes burns**

➢ If the substance or preparation applied to the unbroken healthy skin of an animal causes tissue destruction in all skin layers after an exposure not exceeding four hours, or where such a result may be predicted.

➢ If the substances are organic hydrogen peroxides, except where proven to be safe.

- **Irritant substance or preparation**

Non-corrosive products and preparations are classified as irritant, and characterised by the warning symbol "Xi" and the danger label "irritant".

The corresponding hazard warning is :

- **R38 : irritant to skin**

Substances and preparations causing serious inflammation of the skin, persisting at least 24 hours after a period of exposure not exceeding four hours during a test on rabbits conducted in accordance with *E.E.C. Directive 92/69.*

Skin inflammation is deemed serious if :

-the mean value for erythema and eschar formation, or for oedema, for the whole batch of animals treated is ≥2;
-or, if a test on three animals, conducted in accordance with *E.E.C. Directive 92/69* gives rise to mean erythema and eschar formation or oedema values of ≥ 2 for each animal, in at least two animals.

NOTE :

In both cases, it is preferable to take into account all values obtained at each inspection at 24, 48 and 72 hours and to calculate the respective mean values.

Skin inflammation is also considered serious if it persists in at least two animals at the end of the observation period. Specific effects such as exfoliation, discoloration, fissures, eschars, and alopecia must be taken into consideration.

- Where the substances or preparations cause serious skin inflammation, on the basis of empirical observations in humans.

- If the substances are organic hydrogen peroxides, except where proven to be safe.

7.2 – Interpretation according to French regulations

Values obtained for erythema and œdema, 24, and 72 hours after the product to be tested application, where added for three animals. These figures are divided by six to obtain a Primary Skin irritation index (PSi).

On the basis of this index, primary irritation reactions are interpreted as follows :

PSi	PRODUCT CLASSIFICATION
PSi ≤ 0.5	NON IRRITANT
0.5 < PSi ≤ 2	SLIGHTLY IRRITANT
2 < PSi ≤ 5	IRRITANT
5 < PSi ≤ 8	SEVERELY IRRITANT

- This table is inspired by the classification scheme of Draize *« Dermal toxicity » in : Appraisal of the Safety of chemicals in foods, drugs and cosmetics, Association of food and drug officials of the United States, Austin (Texas)-1959-p46-59.*

8 – AMENDMENTS TO THE PROTOCOL

Modifications to the experimental conditions defined in this protocol are to be recorded as protocol modifications after approval by the Study Director and the Sponsor.

9 – QUALITY ASSURANCE

According to the experimental protocol, the experiment overview and the raw data, the study is inspected by the Quality Assurance Unit of PHYCHER *Bio développement.*

The inspections of the toxicity tests are performed and conducted according to a process for verification of the different major technical phases concerning at least one similar test. Their frequency is once a term or more.

The different inspections are performed in accordance with *the principles of Good Laboratory Practices (G.L.P.) established by the O.E.C.D. decisions, concerning the mutual acceptance of data in the evaluation of chemical substances, (C(81) 30 (final) appendix 2, May 12th, 1981 ; C(97) 186, November 26th, 1997,* and transcribed in the *decree n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française.*

10 – FINAL REPORT

The final report is to contain at least the following information:
- Description of the test product (and vehicle used where applicable);
- The species, strain, sex and number of animals used, together with details of the supplier;
- Type of feed given, and acclimatisation and environmental conditions;
- Study conditions;
- Results of all individual observations, presented in chart form;
- Description of the nature and degree of cutaneous irritation;
- Index of cutaneous irritation and classification of the test product;
- Description of any toxic effects, other than those concerning cutaneous irritation;
- Classification according to current regulations, together with applicable warning symbols and hazard labels.

11 – ARCHIVES

The protocol and amendments to the protocol, original data, correspondence, and final report are to be kept by the company in a dedicated store-room, for a period of ten years.

At the end of this period, the study archives will be either returned to the Sponsor of the study or destroyed.

The test product , from its initial container, is :
- sent back to the Sponsor, at his request, taking into account no storage is conducted,
- generally kept in the original container 3 years after emission of the final report.

PROTOCOL APPROVAL

Date Feby 20th, 2002

[redacted]

Study Director
(Appointed at the start of the study)

MODIFICATION TO THE STANDARD EXPERIMENTAL PROTOCOL

The following modifications will be applied to the standard experimental protocol:

Phycher

Bio développement

ACCORDING TO THE G.L.P.
DESCRIBED IN 88/320 E.E.C. AND
C/81/130 O.E.C.D. DIRECTIVES
BY G.I.P.C. 10/23/98

Assessment of acute irritant/corrosive effect on the eyes

Final Report : IO-OCDE-PH-02/0051

TEST PRODUCT
***Mannostab*^{MD}**
Batch n°10064/06-2000

❑ **STUDY MONITOR** : **ADEC TOX**
[redacted]
120, rue Quintin
F-33000 BORDEAUX

❑ **SPONSOR** : **LAFFORT OENOLOGIE**
Quai de Souys
BP 17
F-33015 BORDEAUX Cedex

Report of 11 pages and 0 appendix ***Cestas, April 02nd, 2002***

CONTENTS

QUALITY ASSURANCE ATTESTATION

I, the undersigned Bernard Benech, Quality Assurance unit of PHYCHER *Bio développement*, attest that the study **IO-OCDE-PH-02/0051** was submitted to the control of Quality Assurance.

The routine inspections of the toxicity tests performed within the laboratory of PHYCHER *Bio développement*, are carried out as a control process of different main technical phases concerning at least one similar test. The frequency is once a term or more. Dates of technical phases inspections relating to this test are given below :

Date of inspection	: February 12th, 2002 *Test product instillation at D0*
Date of reporting to Study Director	: February 12th, 2002
Date of reporting to Management	: February 12th, 2002

This report has been audited by PHYCHER *Bio développement* Quality Assurance unit. It is considered to be an accurate account of the data generated and the application of the operating procedures in use within the laboratory :

	Draft report	Final report
Date of audit report inspection	: March 14th, 2002	April 02nd, 2002
Date of reporting to Study Director	: March 14th, 2002	April 02nd, 2002
Date of reporting to Management	: March 14th, 2002	April 02nd, 2002

Date April 02nd, 2002

[redacted]

AUTHENTICATION

I, the undersigned, François Richeux, Study director, certify that the study **IO-OCDE-PH-02/0051** was performed on the premises of the Laboratory PHYCHER *Bio développement* under the technical responsibility of Corinne Scheyder.

I, certify that the objectives laid down in the technical protocol were achieved and no undesirable event occurred to affect the quality or the integrity of the study. I consider the data generated to be valid. This report fully and accurately reflects the operating procedures used and data generated.

The entire work was devised and performed in compliance with the principles of the Good Laboratory Practices (G.L.P.), as defined in *the O.E.C.D. ruling relative to the mutual acceptance of data in the evaluation of chemical substances (C(81) 30 (final) Appendix 2 - May 12th, 1981; C (97) 186, November 26th, 1997)*, and transcribed in the *decree n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française.*

Date Avril 01-d, 2002

[redacted]

Study Director

SUMMARY AND CONCLUSION OF THE STUDY

The product **Mannostab**[MD] was instilled, as supplied, into the eye of three New Zealand rabbits at the dose of 0.1 g, according to the experimental protocol established on the basis of the official method as defined in the *O.E.C.D. guideline n° 405 dated February 24th, 1987 and the test method B.5 of the E.E.C. directive n° 92/69 dated December 29th, 1992.*

The ocular reactions observed during the study remained slight and only recorded at the conjunctivae level: very slight redness in the 3 animals 1 hour the test product instillation and totally reversible between the 2nd and the 3rd day of the test associated with a lacrymation only at the 1 hour reading time.

In conclusion, the result obtained, in these experimental conditions, enable to conclude that the test product **Mannostab**[MD]:

- is **slightly irritant** for the eye (Max. O.I = **6.7**) according to *the classification established* in *the Journal Officiel de la République Française dated July 10th, 1992.*

- and, **must not be classified** according to the criteria for the classification, packaging and labelling of dangerous substances in compliance with the *E.E.C. Directive n° 67/548 and 93/21.*

Date Avril 02nd 2002

Scientific Direction PHYCHER ~~*Bio développement*~~

[redacted]

Pharmacologist
(Hdr)

ACCREDITATION N° 1-0887

TEST REPORT

1 - TEST PRODUCT

The test product **Mannostab**[MD], sent by ADEC TOX - 120 rue Quintin – F-33000 Bordeaux FRANCE, was received on 02/20/02. Its characteristics were:

- Container : plastic pilular (n=3)
- Quantity : 80.97g (container + contents)
- Batch : 10064/06-2000
- Form : powder
- Colour : whitish
- Storage : room temperature

It was identified under the code number: **PH-02/0051**.

Informations concerning the identity, purity and stability of the test product are the responsibility of the Sponsor. An information data sheet concerning the product was provided by the study monitor.

2 – STUDY DEVELOPMENT

The study was carried out between 02/25/2002 and 03/01/2002, in *PHYCHER Bio développement - ZI de Toctoucau - 18 chemin de Lou Tribail - 33611 Cestas Cedex*, according to the *protocol Ref. IO-O.C.D.E. - version September 1999,* established according to the *O.E.C.D. guideline n° 405 dated February 24th, 1987 and the test method B.5 of the E.E.C. directive n° 92/69 dated December 29th, 1992.*

2.1 - Animals

Three female albinos rabbits of New Zealand strain, numbered A4537, A4499 and A4500, originating from the Elevage de Gérome (Quartier Labaste – F40260 Linxe) were kept during a 6 to 7-day acclimatisation period. During the test, the animals weighed between 2.21kg and 2.80kg.

Animals were kept in individuals boxes, the environmental conditions were :

- temperature : between 20 °C and 22 °C
- relative humidity : between 46% and 55%

2.2 - Treatment

A volume of 0.1 g of the pure test product was instilled into one eye of each rabbit, the other eye untreated serving as control.

2.3 - Grading of the reactions

Ocular examinations were performed on both right and left eyes 1 hour after instillation and 24, 48 and 72 hours later.

NOTE :

If no reaction is observed at 72 hours (D3) after instillation, the study is finished. In case of persistent reactions, additional observations can be carried out at Day 4 (D4), to Day 21 (D21) in order to determine the reversible character of the lesions observed.

3 - RESULTS

The individual and mean scores obtained during the study are shown in tables 1 to 5 hereafter.

The ocular reactions observed during the study remained slight and only recorded at the conjunctivae level: very slight redness in the 3 animals 1 hour the test product instillation and totally reversible between the 2nd and the 3rd day of the test associated with a lacrymation only at the 1 hour reading time.

4 - CONCLUSION

The result obtained, in these experimental conditions, enable to conclude that the test product **Mannostab**MD:

- is **slightly irritant** for the eye (Max. O.I = **6.7**) according to *the classification established* in *the Journal Officiel de la République Française dated July 10th,1992.*

- and, **must not be classified** according to the criteria for the classification, packaging and labelling of dangerous substances in compliance with the *E.E.C. Directive n° 67/548 and 93/21.*

5 - ARCHIVES

All the original data related and the final report related to this study will be stored for a period of 10 years in the company under the reference **IO-OCDE-PH-02/0051**. After this period, the Sponsor's instructions will be applied.

6 - PROTOCOL ADHERENCE

No deviation was registered during this test.

Date 03.01.2002

Technical Responsible

[redacted]

ASSESSMENT OF IRRITANT/CORROSIVE EFFECT ON THE EYES

TEST PRODUCT: Mannostab^{MD}

Instillation: 0.1g of pure test product

Operator: [redacted]

Instillation dates (D0): 02/25/2002 (A4537)
02/26/2002 (A4499 & A4500)

Number of animals: 3

Table 1

INDIVIDUAL AND MEAN SCORES OF CONJUNCTIVA, IRIS AND CORNEA

(In compliance with the E.E.C. Regulations)

Animal n° *(Weight kg)*	OBSERVATION TIME	CONJUNCTIVA		IRIS	CORNEA
		CHEMOSIS (A)	REDNESS (C)	LESION (D)	OPACITY (E)
A4537 *(2.21)*	24 hours	0	1	0	0
	48 hours	0	0	0	0
	72 hours	0	0	0	0
TOTAL		0	1	0	0
Mean		0.0	0.3	0.0	0.0
A4499 *(2.70)*	24 hours	0	0	0	0
	48 hours	0	0	0	0
	72 hours	0	0	0	0
TOTAL		0	0	0	0
Mean		0.0	0.0	0.0	0.0
A4500 *(2.70)*	24 hours	0	1	0	0
	48 hours	0	0	0	0
	72 hours	0	0	0	0
TOTAL		0	1	0	0
Mean		0.0	0.3	0.0	0.0
CLASSIFICATION		***According to the calculated mean, the product should not be classified, in accordance with the European regulation.***			

ASSESSMENT OF IRRITANT/CORROSIVE EFFECT ON THE EYES

TEST PRODUCT: Mannostab[MD]

Instillation: 0.1 g of pure test product

Operator: [redacted]

Instillation dates (D0): 02/25/2002 (A4537)
02/26/2002 (A4499 & A4500)

Number of animals: 3

Table 2

DETERMINATION OF OCULAR IRRITATION INDICES

(In compliance with the Journal Officiel de la République Française)

Observation time	TOTAL INDIVIDUAL DATA						TOTAL SCORE	Average irritation index
	Animal n°	Weight (kg)	Animal n°	Weight (kg)	Animal n°	Weight (kg)		
	A4537	Start: 2.21 End: 2.23	A4499	Start: 2.70 End: 2.77	A4500	Start: 2.70 End: 2.80		
1 Hour	6		6		8		20	6.7
Day 1 (D1)	2		0		2		4	1.3
Day 2 (D2)	0		0		0		0	0.0
Day 3 (D3)	0		0		0		0	0.0
MAXIMUM OCULAR IRRITATION INDEX (Max. O.I.)								6.7
CLASSIFICATION according to the classification established in the Journal Officiel de la République Francaise dated July 10th, 1992							***Slightly irritant***	

ASSESSMENT OF IRRITANT/CORROSIVE EFFECT ON THE EYES

TEST PRODUCT: Mannostab[MD]

Instillation: 0.1g of pure test product

Operator: [redacted]

Instillation date (D0): 02/25/2002

Animal n°: A4537

Table 3

TOTAL AND INDIVIDUAL SCORES OF OCULAR IRRITATION

Observation time	CONJUNCTIVA				IRIS		CORNEA			Individual irritation index
	A	B	C	(A+B+C)*2	D	D*5	E	F	E*F*5	
				X=		Y=			Z=	X+Y+Z=
1 Hour	0	2	1	**6**	0	**0**	0	0	**0**	**6**
Day 1 (D1)	0	0	1	**2**	0	**0**	0	0	**0**	**2**
Day 2 (D2)	0	0	0	**0**	0	**0**	0	0	**0**	**0**
Day 3 (D3)	0	0	0	**0**	0	**0**	0	0	**0**	**0**

ASSESSMENT OF IRRITANT/CORROSIVE EFFECT ON THE EYES

TEST PRODUCT: Mannostab^{MD}

Instillation: 0.1g of pure test product Operator: [redacted]

Instillation date (D0): 02/26/2002 Animal n°: A4499

Table 4

TOTAL AND INDIVIDUAL SCORES OF OCULAR IRRITATION

Observation time	CONJUNCTIVA				IRIS		CORNEA			Individual irritation index
	A	B	C	(A+B+C)*2	D	D*5	E	F	E*F*5	
				X=		Y=			Z=	X+Y+Z=
1 Hour	0	2	1	**6**	0	**0**	0	0	**0**	**6**
Day 1 (D1)	0	0	0	**0**	0	**0**	0	0	**0**	**0**
Day 2 (D2)	0	0	0	**0**	0	**0**	0	0	**0**	**0**
Day 3 (D3)	0	0	0	**0**	0	**0**	0	0	**0**	**0**

ASSESSMENT OF IRRITANT/CORROSIVE EFFECT ON THE EYES

TEST PRODUCT: Mannostab[MD]

Instillation: 0.1g of pure test product

Operator: [redacted]

Instillation date (D0): 02/26/2002

Animal n°: A4500

Table 5

TOTAL AND INDIVIDUAL SCORES OF OCULAR IRRITATION

Observation time	CONJUNCTIVA				IRIS		CORNEA			Individual irritation index
	A	B	C	(A+B+C)*2	D	D*5	E	F	E*F*5	
				X=		Y=			Z=	X+Y+Z=
1 Hour	0	3	1	**8**	0	**0**	0	0	**0**	**8**
Day 1 (D1)	0	0	1	**2**	0	**0**	0	0	**0**	**2**
Day 2 (D2)	0	0	0	**0**	0	**0**	0	0	**0**	**0**
Day 3 (D3)	0	0	0	**0**	0	**0**	0	0	**0**	**0**

Phycher
Bio développement

STUDY ORGANISATION

EVALUATION OF ACUTE IRRITANT/CORROSIVE EFFECTS ON THE EYES

References : *O.E.C.D. Guideline n° 405 dated February 24th 1987*
E.E.C. Directive n° 92/69 dated July 31st 1992 (E.E.C.J. L383 A dated December 29th 1992)

Test will be performed under the experimental protocol IO-OCDE - version September 1999.

Test product : Mannostab[MD] **Batch** : 10064/06-2000

Sponsor :
Laffort Oenologie
Quai de Souys
BP 17
33015 BORDEAUX Cedex

Study monitor:
ADEC TOX
[redacted]
120, rue quintin
33000 BORDEAUX

Testing facility : PHYCHER *Bio Développement*
18 chemin de Lou Tribail
ZA de Toctoucau
33610 CESTAS

Study director : [redacted]

Schedule study:

Start of study :	Week 10*	
End of study:	Week 12	Results by fax
DRAFT :	Week 14	-
Final report:	Week 15	2 reports (english)

* If signing of the study organization by the study monitor week 08

Approbation

Date : Feb 21st 2002
[redacted]
Study director

Date : 1.3.02
[redacted]

EXPERIMENTAL PROTOCOL
REF: IO-O.C.D.E.

Version september 1999

EVALUATION OF ACUTE IRRITANT/CORROSIVE EFFECT ON THE EYES

References:

- ***OECD Guideline n° 405 dated February 24th, 1987***
- ***EEC Directive n° 92/69 dated July 31st, 1992 (E.E.C. Journal L383 A dated December 29th, 1992)***

1 - INTRODUCTION

➢ This protocol has been devised in accordance with:

- *OECD Guideline n$_o$ 405 dated February 24th, 1987 and,*

- *EEC Directive n$_o$ 92/69 dated July 31st 1992 (EEC Journal L383 A dated December 29th, 1992), relating to the evaluation of acute irritant/corrosive effects of chemical substances on the eyes.*

➢ Before any test, an experiment overview dated and signed by the study director is established and submitted to the management in order to define the object of the study and its development.

These documents are applied and used in compliance with the Principles of the Good Laboratory Practices (G.L.P.).

2 - PRINCIPLE

The test product is instilled into the eyes of three rabbits in a single dose. Each rabbit is its own control. The degree of irritation is assessed at set intervals.

The test is conducted over a sufficient length of time, at least six days, in order to determine the reversibility or irreversibility of the effects observed. There is generally no need to exceed 21 days post-application.

All available information concerning the product to be tested should be taken into consideration in order to limit tests liable to produce severe reactions, such as:

- ***Acidic products of pH 2 or less, and alkaline products of pH 11.5 or greater with corrosive properties;***
- ***Products shown to be highly toxic when administered by the cutaneous route;***
- ***Products for which in vitro tests suggest the likelihood of irritant, severely irritant or corrosive properties. In this case, a reduced test will be performed after an advice of the toxicologist expert.***

NOTES :

In the cases mentioned above, the test may not need to be performed, a single animal will be used to confirm the predicted severe effects. In the total absence of information, the test is to begin with a single animal.

3 - ANIMALS USED AND HOUSING CONDITIONS

3.1 - Animals

Three albino New Zealand rabbits of the same sex, male or female, aged approximately 11 weeks at the start of the test, and generally weighing between 2.2 and 2.5 kg at the start of the test, are to be used.
Where results are not conclusive, a larger number of animals may be required, after approval by the Sponsor.

3.2 - Housing

The animals are housed in individual wire floored stainless steel cages of dimensions 61 cm x 46 cm x 34 cm. The door of each cage is fitted with a feeding dish and a drinking device of 1.000ml.

The cages are placed in an air-conditioned animal holding facility :

- Air recycling: at least 10 cycles per hour,
- Temperature: 20° C ± 3° C,
- Relative humidity: from 30 % to 70 %,

which circadian cycle : 12 hrs day / 12 hrs darkness.

The extreme values for the temperature and the relative humidity during the test are included in the final report.

3.3 – Food and drink

Drinking water (tap-water from public distribution system) and foodstuff (UAR 112 from UAR in 91360 Villemoisson s/Orge, France) are supplied freely.
Microbiological and chemical analyses of the water are carried out once every six months by the Institut Européen de l'Environnement de Bordeaux (I.E.E.B.).

4 - OPERATING PROCEDURE

4.1 - Preparation of the animals

Prior to the test, the animals are to be kept during an acclimatisation period of at least 5 days, under the same living and feeding conditions as for the test.

They are to be identified on the day of arrival by a numbered metal ring (Chevillot-Quick) attached to the side of the ear.

Prior to instilling the product, the rabbits' eyes are to be examined to exclude any displaying signs of ocular lesions.

4.2 - Characteristics of the test product

The test product is to be coded upon reception by the laboratory, using the operating procedure. The characteristics of the product (conditioning, quality, form, colour) are to be logged within the data base form and completed if necessary, upon opening the sample in the experimental laboratory.

The test product is subsequently stored in a suitable place in the laboratory at room temperature or 4°C in the fridge in accordance with information applied by the Sponsor.

All available information (safety data sheet) on the product under investigation should be made available to the laboratory. Information concerning the identity, purity and the stability of the test product are the responsibility of the Sponsor. An analysis report or certificate for the sample under investigation is requested from the Sponsor who ordered the test and enclosed in the final report.

4.3 - Treatment

The animals are to be immobilised on a restraining table, and the test product applied directly to the lower conjunctival sinus of one of each rabbit's eyes, as follows :

- *for liquid and semi-liquid products: 0.1ml using a 1 ml syringe with 1/10 markings,*
-
- *for pasty or powdery products: a volume of 0.1ml, without exceeding a mass of 100mg,*

- *for solid or granular products: a volume of 0.1ml after grinding into a fine powder,*

- *for products supplied as a spray or pressurised aerosol: 0.1ml after collecting contents or a one-second spray 10 cm from the eye, which is to be held open. The dose applied may be estimated by spraying the aerosol onto a sheet of paper card, through a 1 cm x 1 cm hole. The increase in the weight of the blotting paper gives an approximate indication of the quantity administered. Care should be taken not to damage the eye.*

Depending on the conditions of use of the test product, and/or its texture, a modified preparation may prove necessary. In this case, the vehicle influence is taken into consideration and this will be studied with a new group of 3 rabbits with approval of the Sponsor.

The animals' eyelids are to be held closed for about 10 seconds, to prevent any of the test product from escaping. The animals are kept on a restraining table for an hour after the initial examination, then replaced in their cage.

NOTES:

- *Where the product is suspected of producing a severe irritant or corrosive effect, an initial test is to be planned with a single animal, in the same experimental conditions described above.*

- *The eyes are not to be rinsed during the 24 hours following the instillation of the product to be tested. After 24 hours, the eyes may be rinsed, if necessary.*

- *Where the products under evaluation prove too irritant, an additional test, initially with one rabbit, may be conducted, with the eye being rinsed approximately 30 seconds after instillation. This rinse should be abundant (30 seconds) and must not cause any lesions. If results prove satisfactory, the test may be extended to three rabbits.*

- *If a corrosive irritant effect, instantaneous or 1 hour after the instillation, is observed the test is immediately interrupted.*

5 - MACROSCOPIC EXAMINATIONS AND EVALUATION OF OCULAR IRRITATION

Examinations of the treated eye and the control eye are made 1 hour, then 24, 48 and 72 hours after instillation, then 4 to 7 days, if necessary, or even 14 or 21 days.

Observations are to be carried out under identical conditions, in particular as regards ambient lighting conditions, and in the same order as for the instillation.

Eye examinations are carried out using the scale of lesion scores published *in E.E.C. Directive n° 92/69 dated July 31st, 1992*, and in the following order :

5.1 - Conjunctiva

Chemosis and lachrymation are to be assessed prior to any forced opening of the eyelids.

CHEMOSIS

- No swelling **0**
- Slight swelling, including the nictitating membrane **1**
- Swelling with eversion of the eyelid **2**
- Swelling with eyelid half-closed **3**
- Swelling with eyelid more than half-closed **4**

SCORE OBTAINED (A)

LACHRYMATION

- No lachrymation **0**
- Slight lachrymation (normal slight secretions in the inner corner not to be
- taken into account) **1**
- Lachrymation with moistening of the eyelids and neighbouring hairs **2**
- Lachrymation with moistening of the eyelids and large areas around the eye **3**

SCORE OBTAINED (B)

REDNESS OF THE EYES

- Blood vessels normal **0**
- Vessels significantly more prominent than normal **1**
- Vessels individually distinguishable with difficulty
  - Generalised red coloration **2**
  - Generalised deep red coloration **3**

SCORE OBTAINED (C)

5.2 - Iris

This examination is performed by shining an electric torch (or ophtalmoscope) directly into the eye:

- Normal **0**
- Iris significantly more wrinkled than normal, congestion,
  swelling of the iris which continues to react to light, even slowly **1**
- No reaction to light, haemorrhage, significant damage
  (any or all of these characteristics) **2**

SCORE OBTAINED (D)

5.3 - Cornea

In order to confirm the presence or absence of corneal lesions, a direct examination is to be performed to assess the degree and extent of opacification and or any epithelial effects.
This examination is to be completed, by an examination, after an instillation of a 2% solution of sodium fluorescein, with the exception of the reading at 1 hour.
Excess fluorescein is to be eliminated by flushing with physiological serum, taking care to avoid any lesions.

Only the area worst subjected to lesions is to be taken into account.

DEGREE OF CLOUDING

- No modification visible either directly or after instillation of fluorescein (no loss of glint or polish) **0**
- Translucent areas (diffuse or disseminated), iris details clearly visible **1**
- Easily identifiable translucent area, iris details slightly obscured **2**
- Opalescent area, no iris details visible, pupil outline scarcely distinguishable **3**
- Total corneal opacity, completely obscuring the iris and pupil **4**

SCORE OBTAINED (E)

EXTENT OF OPACITY

- Opaque area present but covering one quarter or less **1**
- Between one quarter and half **2**
- Between half and three quarters **3**
- Between three quarters and the entire surface **4**

SCORE OBTAINED (F)

NOTES:

- *If no irritation persists after 72 hours, the test is to be concluded. Where the reverse is true, the observation period may be extended to the 7th or 21st day, in order to monitor changes in the lesions and determine their reversibility or irreversibility.*
- *Where the choice between two values is unclear, the higher value is to be chosen.*

6 - EXAMINATION OF THE GENERAL CONDITION OF THE ANIMALS

The systemic toxicity, expressing by behavioural abnormalities, alteration of neuro-vegetative reactions or decrease feeding, and any toxic effects are to be logged and described.
The animals are weighed at the start and at the end of the test.

7 - INTERPRETATION OF THE RESULTS

7.1 - Interpretation according to European regulations

In accordance with *E.E.C. Directives 93/21 (J.O.C.E. L110 dated May 4th, 1993), 91/325 dated March 5th, 1991 (J.O.C.E. L180 dated July 8th, 1991) and 67/548*, relating to the labelling, packaging and classification of dangerous substances, mean values are to be calculated for observations made at 24, 48 and 72 hours. This mean enables products may to be classified as follows :

♦ **Irritant substance or preparation :**

Non-corrosive substances and preparations are classed as irritant, and represented by the warning symbol *"Xi"* and the danger label *"irritant"*.

The corresponding hazard warning is :

- **R36 : irritating to eyes**

➢ If the substances and preparations cause significant ocular lesions to the eye of the animal, which form during the 72 hours following instillation and persist for at least 24 hours.

The ocular lesions are considered as important.

◆ If the mean values for the test when conducted according to E.E.C. Directive $n_0$92/69 is :

- ≥ 2 but < 3 for corneal opacity,
- ≥ 1 but ≤ 1.5 for lesions of the iris,
- ≥ 2.5 for redness of the conjunctiva,
- ≥ 2 for conjunctival oedema (chemosis).

◆ If the test conducted on three rabbits in accordance with E.E.C. Directive n_0 92/69 causes lesions on at least two animals with mean values as stated above, with the exception of :

- lesions of the iris, where the value must be ≥ 1 but < 2
- redness of the eye, where the value must be ≥ 2.5

NOTE:

In the two situations mentioned above, all values obtained at each examination (24, 48 and 72 hours) must be included in the calculation of the respective means.

➢ If the products and preparations cause significant ocular lesions, on the basis of empirical observations in humans.

➢ If the products are organic peroxides, except where proven to be safe.

- **R41 : risk of serious damage to eyes**

➢ If the products and preparations cause significant ocular lesions to the eye of the animal, which form during the 72 hours following instillation and persist for at least 24 hours.

Ocular lesions are to be considered severe.

◆ If the mean values for the ocular irritation test when conducted according to E.E.C. Directive $n_0$92/69 is :

- ≥ 3 for corneal opacity,
- >1.5 for lesions of the iris.

◆Where the test conducted on three rabbits in accordance with E.E.C. Directive n_0 92/69, if the lesions observed in at least two animals produce one of the following mean values :

- ≥ 3 for corneal opacity,
- = 2 for lesions of the iris.

NOTE :

In the two situations mentioned above, all the values obtained at each examination (24, 48 and 72 hours) must be included in the calculation of the respective means.

Ocular lesions are also deemed to be severe if they persist until the end of the observation period.

Ocular lesions are also considered to be severe if the product or preparation produces an irreversible colouring of the eye.

➢ If the products and preparations cause severe ocular lesions, on the basis of empirical observations in humans.

NOTE :

When a product or preparation is classified as corrosive with the hazard warning R34 or R35, the risk of the severe ocular lesions is considered as tacit and the hazard warning R41 is not included on the labelling.

7.2 - Interpretation according to French regulations

The following calculations are performed for each animal and each observation period :

- Conjunctiva : $X = 2 \times (A + B + C)$ $(X \leq 20)$
- Iris : $Y = 5 \times D$ $(Y \leq 10)$
- Cornea : $Z = 5 \times E \times F$ $(Z \leq 80)$

The resulting figures are compatible with the *classification system developed by J.H. Kay and J.C. Calandra, (J. Soc. Cosmet., 1962 –13 p. 281-289), together with the Journal Officiel de la République Française dated July 10th 1992 (order dated June 9th 1992).* This system allows the ocular irritation of products under assessment to be classified. All of the values obtained for the conjunctiva, the iris and the cornea of each animal are calculated. The highest mean value (O.I. max) is used to classify the product on trial, according to the scale published by the *Journal Officiel de la République Française*, and reproduced here :

O.I. max	PRODUCT CLASSIFICATION
≤ 15	SLIGHTLY IRRITANT
> 15 and ≤ 30	MODERATELY IRRITANT
>30 and ≤ 50	IRRITANT
> 50	HIGHLY IRRITANT

8 - PROTOCOL AMENDMENTS

Modifications to the experimental conditions defined in this protocol are to be recorded as protocol modifications after approval by the Study Director and the Sponsor.

9 - QUALITY ASSURANCE

According to the experimental protocol, the experiment overview, the raw data of the study are inspected by the Quality Assurance unit of PHYCHER *Bio Développement.*

The inspection of the toxicity tests are performed and conducted according to a process for verification of the different major technical phases concerning at least one similar test. The frequency is once a term or more.

The different inspections are performed in accordance with *the principles of Good Laboratory Practices (G.L.P.) established by the O.E.C.D. decisions, concerning the mutual acceptance of data in assessment of chemical substances, (C (81) 30 (final) appendix 2, may 12th, 1981; C(97) 186, November 26th, 1997),* and transcribed in the *decree n° 98-1312 dated December 31st, 1998 of the Journal Officiel de la République Française.*

10 - FINAL REPORT

The final report is to contain at least the following information:

- Description of the test product (and excipient where applicable),
- The species, breed, sex and number of animals used, together with details of the supplier,
- Type of feed given, and acclimatisation and environmental conditions,
- Test conditions,
- Results of all individual observations, presented in chart form,
- Description of the nature and degree of ocular irritation,
- Index of ocular irritation and classification of the product,
- Description of any toxic effects, other than those concerning ocular irritation,
- Classification according to current regulations, together with applicable warning symbols and hazard labels (if necessary).

11 - ARCHIVES

The protocol and protocol modifications, original data, correspondence, and final report are to be kept by the company in a dedicated store-room, for a period of ten years.

At the end of this period, the study archives will be either returned to the Sponsor of the study or destroyed.

The test product , from its initial container, is :
- sent back to the Sponsor, at his request, taking into account no storage is conducted,
- generally kept in the original container 3 years after emission of the final report.

PROTOCOL APPROVAL

Date: Feb 21st, 2002

[redacted]

Study Director
(Appointed at the start of the study)

MODIFICATION TO THE STANDARD EXPERIMENTAL PROTOCOL

The following modifications will be applied to the standard experimental protocol: