

**CONFIDENTIAL**

ETAP CODE N°: INGREDIA 01/0399/ING 913/IRWIN TEST/AM

INGREDIA CODE N°: ING 913/ITAM/R-IP

**ASSESSMENT OF BEHAVIORAL EFFECTS OF "ING 913" PEPTIDE**  
*Primary observation in male Wistar rats on the morning*

**FINAL REPORT**

**SPONSOR**

INGREDIA

51 - 53, Avenue F. Lobbedez B.P. 946  
62033 ARRAS CEDEX - FRANCE

**AUTHORS**

M. MESSAOUDI  
F. DESOR  
D. DESOR

**Representative**

J.-F. BOUDIER  
B. DEMAGNY

Study period: April 27 - 30, 1999

Draft report date: September 7, 1999

Final report date: December 7, 1999

**ETAP - Ethologie Appliquée**

Siège Social : 1, rue Blaise Pascal F-54320 MAXEVILLE - Tél. : 03 83 95 53 00 - Fax : 03 83 98 07 54  
Centre de Recherches : 40, rue Lionnois F-54000 NANCY - Tél. : 03 83 37 26 74 - Fax : 03 83 37 84 77

E-mail : etap@etap-lab.com

S.A. au Capital de 300 000 F - R.C.S. Nancy - Siret : 380 676 767 00011

**CONTENTS**

**QUALITY ASSURANCE STATEMENT** ..... 2

**AUTHORS** ..... 3

**1 - SUMMARY** ..... 4

**2 - INTRODUCTION** ..... 5

**3 - MATERIALS & METHODS** ..... 5

    3.1 - Animals ..... 5

    3.2 - Product ..... 5

    3.3 - Procedure ..... 5

    3.4 - Administration of the product ..... 6

    3.5 - Variables ..... 6

    3.6 - Statistics ..... 6

**4 - RESULTS** ..... 7

    4.1 - Startle response ..... 7

    4.2 - Touch escape ..... 7

    4.3 - Tail pinch ..... 7

    4.4 - Irritability ..... 7

    4.5 - Other tests and safety ..... 8

**5 - CONCLUSION** ..... 11

**6 - REFERENCES** ..... 12

**7 - ARCHIVES STATEMENT** ..... 12

**8 - APPENDICES** ..... 13

    8.1 - Appendix 1: Study time plan ..... 13

    8.2 - Appendix 2: Individual results ..... 14



**QUALITY ASSURANCE STATEMENT**

STUDY: ETAP code N°: Ingredia-01/0399/ING 913/Irwin test/AM  
Ingredia code N°: ING 913/ITAM/R-IP

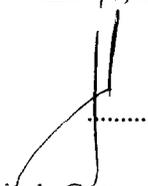
TITLE: Assessment of behavioral effects of "ING 913" peptide:  
Primary observation in male Wistar rats on the morning.

I, the undersigned, hereby declare that the results presented in this report, in some cases recorded automatically and in others transcribed from the original data sheets, were verified by me item by item in comparison with the original data sheets.

To the best of my knowledge, there were no circumstances that may have affected the quality or integrity of the data.

December 7, 1999

Pr. D. DESOR, Scientific Adviser and Quality Assurance  
Ethopharmacology - Biostatistics  
Université Henri Poincaré - Laboratoire de Biologie et Physiologie du Comportement  
U.R.A. C.N.R.S. 1293 - 54500 Vandœuvre-lès-Nancy.  
Government authorization to perform experiments on live animals n° 04140/1991.



**AUTHORS**

I, the undersigned, hereby declare that the work described in this report was performed under my supervision as Scientific Director and that the report provides a true and accurate record of the results obtained.

I declare further that the present study was performed in accordance with ETAP's Standard Operating Procedures and in accordance with the principles of Good Laboratory Practice, including appropriate archiving of the original data sheets.

	Signature	Date
M. MESSAOUDI, Scientific Director Biology of Behavior - Ethopharmacology Government authorization to perform experiments on live animals n° 04535/1991		December 7, 1999

We the undersigned, responsible for the execution of the experiments described in this report hereby, declare that the experiments were performed as described and that the data presented correspond exactly to the results obtained during the experiments.

	Signature	Date
F. DESOR, Research Technician Ethopharmacology Authorization to perform experiments on live animals		December 7, 1999

The present study was carried out in ETAP's Research Center  
40, rue Lionnois F-54000 Nancy - Government authorization n° B54805



**1 - SUMMARY**

Behavioral effects of ING 913 peptide were assessed on the morning using Irwin's test in male Wistar rats. The drug was administered at the doses of 0.5, 1.5 and 4.5 mg/kg, i.p., 30 minutes before the first observation. Control rats received saline solution and were tested in the same conditions.

In the following study, the characteristics of ING 913 that appear are in favor of an antistress profile which is apparent from:

- decreased reaction to startle at 4.5 mg/kg;
- decreased reaction to touch escape at 0.5, 1.5 and 4.5 mg/kg;
- decreased reaction to tail pinch at 0.5, 1.5 and 4.5 mg/kg.

At the dose of 4.5 mg/kg, ING 913 induced irritability at 30, 60 and 120 minutes after the treatment administration.

The safety profile is satisfactory.

## 2 - INTRODUCTION

ETAP-Ethologie Appliquée was asked by INGREDIA S.A. to investigate the behavioral effects of the peptide "ING 913", administered on the morning at the doses of 0.5, 1.5 and 4.5 mg/kg, i.p., diluted in 0.9% NaCl solution, using a study method derived from that of Irwin (1968) in male Wistar rats.

## 3 - MATERIALS & METHODS

### 3.1 - Animals

Twelve male Wistar rats (Centre d'élevage Iffa Credo, L'Arbresle - France), weighing 260-280 g at the start of experiment, were used for the study. They were housed in groups of three in a climate controlled room with a 12-h light:dark cycle (light off at 8 a.m.) with access to food and water *ad libitum*.

After seven day acclimatization period, following their entry in laboratory, the rats were weighed, identified and then randomly assigned to one of the four treatment groups (n = 3).

### 3.2 - Product

**Table 1**  
**Product**

Product	ING 913	Saline
Origin	Génosphère France	B.Braun Fandre France
Batch number	6986	426350
Expiry date	-	12/2003
Preparation	Diluted in 0.9% NaCl solution	-
Storage	At -20° C. protected from the light	At 4°C. protected from the light

### 3.3 - Procedure

The rats were injected at 8.30 a.m. with the "ING 913" peptide solutions and then observed in comparison with control ones given vehicle. Observations were performed 30, 60 and 120 minutes after administration of the peptide and also 24 hours later for delayed death.

### 3.4 - Administration of the product

The product "ING 913" was dissolved in 0.9% NaCl solution on a magnetic stirrer for 30 minutes. The solutions were prepared just before their i.p. administration, 30 minutes prior to the primary observation test (Tab. 2).

**Table 2**  
Administration of the product

Group	Rats per group	Treatment	Dose (mg/kg, i.p.)	Volume ml/kg	Administration before test (minutes)
Control	3	Saline	-	2	30
Group 1	3	ING 913	0.5	2	30
Group 2	3	ING 913	1.5	2	30
Group 3	3	ING 913	4.5	2	30

### 3.5 - Variables

The following items concerning behavioral changes, but also physiological and toxic symptoms and mortality were recorded:

- Behavior:

- spontaneous activity;
- motor affective response;
- sensorimotor response.

- Neurology:

- posture;
- muscle tone;
- equilibrium and gait;
- CNS excitement.

- Autonomic NS:

- eyes;
- secretions and excretions;
- miscellaneous.

- Toxicology:

- mortality.

### 3.6 - Statistics

Only for information, Mann-Whitney U-test was used to compare ING 913-treated rats to control ones.

## 4 - RESULTS

Tables 3a and 3b showed all the assessed behavioral effects of the peptide "ING 913", observed at 30, 60 and 120 minutes after its administration at the doses of 0.5, 1.5 and 4.5 mg/kg, i.p., in male Wistar rats.

### 4.1 - Startle response

This is defined as a sudden body jerking movement of the animal in response to a noisy tool snap. It is increased by fearfulness or increasing CNS excitability.

ING 913-treated rats, at the dose of 4.5 mg/kg, showed less reaction than control ones at 30 minutes after the treatment administration.

### 4.2 - Touch escape

This is defined as response of the animal, when it is grasped. It is scored in terms of the vigor of the escape.

ING 913-treated rats, at the doses of 0.5, 1.5 and 4.5 mg/kg, showed less reaction than control ones at 60 and 120 minutes after the treatment administration.

### 4.3 - Tail pinch

This is defined as response of the animal, after first establishing its pattern of movement, when moderate forceps pressure is applied about 2.5 cm above the base of the tail for about 4 sec. It is scored in terms of the vigor of the response (escape, biting, vocalization or freezing).

ING 913-treated rats, at the doses of 0.5, 1.5 and 4.5 mg/kg, showed less reaction than control ones at 120 minutes after the treatment administration.

### 4.4 - Irritability

This is the attempted nipping or biting behavior exhibited by the animals when tested for body tone or grasped and lifted by the scruff of the neck for positional passivity and abdominal tone testing.

ING 913-treated rats, at the dose of 4.5 mg/kg, showed more irritability than control ones at 30, 60 and 120 minutes after the treatment administration.

**4.5 - Other tests and safety**

Tables 3a and 3b list all the tests performed. Only those where treated animals showed modified responses have been detailed above.

In particular there were no observed anomalies of skin color. There was no bizarre behavior, no tremors, no twitching, no convulsions and no episodes of ataxia. There was no diarrhea, no effect on pupil diameter and no exophthalmos.

From the safety point of view no animal died during observation and all were sacrificed after 24 hours.

**Table 3a**  
Behavioral effects of the peptide "ING 913"

Time	30 minutes	60 minutes	120 minutes
Body Position	N.S.	N.S.	N.S.
Bizarre Behaviour	None	None	None
Exophthalmos	None	None	None
Respiratory Rate	N.S.	N.S.	N.S.
Tremors	None	None	None
Twitches	None	None	None
Convulsions	None	None	None
Transfer Arousal	N.S.	N.S.	N.S.
Spatial Locomotion	N.S.	N.S.	N.S.
Palpebral Closure	N.S.	N.S.	N.S.
Startle Response	* Less reaction at 4.5 mg/kg	N.S.	N.S.
Piloerection	None	None	None
Alley Progression	N.S.	N.S.	N.S.
Pelvic Elevation	N.S.	N.S.	N.S.
Tail Elevation	N.S.	N.S.	N.S.
Finger Approach	N.S.	N.S.	N.S.
Finger Withdrawal	N.S.	N.S.	N.S.
Touch Escape	N.S.	* Less reaction at 0.5 mg/kg 1.5 mg/kg 4.5 mg/kg	* Less reaction at 0.5 mg/kg 1.5 mg/kg 4.5 mg/kg
Ataxic Gait	N.S.	N.S.	N.S.
Hypotonic Gait	N.S.	N.S.	N.S.
Impaired Gait	N.S.	N.S.	N.S.
Limb Rotation	N.S.	N.S.	N.S.
Total Gait Incapacity	N.S.	N.S.	N.S.
Positional Passivity	N.S.	N.S.	N.S.

\* p < 0.05; N.S., non significant (Mann-Whitney U-test: ING 913 vs. Control).

**Table 3b**  
**Behavioral effects of the peptide "ING 913"**

Time	30 minutes	60 minutes	120 minutes
Visual Placing	N.S.	N.S.	N.S.
Grip Strength	N.S.	N.S.	N.S.
Body Tone	N.S.	N.S.	N.S.
Pinna	N.S.	N.S.	N.S.
Palpebral Reflex	N.S.	N.S.	N.S.
Toe Pinch	N.S.	N.S.	N.S.
Position Struggle	N.S.	N.S.	N.S.
Wire Manoeuvre	N.S.	N.S.	N.S.
Skin Color	N.S.	N.S.	N.S.
Erection / Ejaculation	None	None	None
Diarrhea	None	None	None
Limb Tone	N.S.	N.S.	N.S.
Abdominal Tone	N.S.	N.S.	N.S.
Pupil Size	N.S.	N.S.	N.S.
Light Pupil Response	N.S.	N.S.	N.S.
Lacrimation / Salivation	None	None	None
Provoked Biting	None	None	None
Tail Pinch	N.S.	N.S.	* Less reaction at 0.5 mg/kg 1.5 mg/kg 4.5 mg/kg
Righting Reflex	N.S.	N.S.	N.S.
Temperature	N.S.	N.S.	N.S.
Irritability	* More reaction at 4.5 mg/kg	* More reaction at 4.5 mg/kg	* More reaction at 4.5 mg/kg
Vocalizations	N.S.	N.S.	N.S.
Urination / Defecation	N.S.	N.S.	N.S.
Provoked Freezing	N.S.	N.S.	N.S.
Delayed / Acute Death	None	None	None

\*  $p < 0.05$ ; N.S., non significant (Mann-Whitney U-test: ING 913 vs. Control)

## 5 - CONCLUSION

Behavioral effects of the product "ING 913" were assessed on the morning using Irwin's test in male Wistar rats. The drug was administered at the doses of 0.5, 1.5 and 4.5 mg/kg, i.p., 30 minutes before the first observation. Control rats received saline solution and were tested in the same conditions.

In our experimental conditions, the characteristics of ING 913 that appear are in favor of an antistress profile which is apparent from decreased reaction to startle, to touch escape and decreased reaction to tail pinch.

At the dose of 4.5 mg/kg, ING 913 induced irritability at 30, 60 and 120 minutes after the treatment administration.

The safety profile is satisfactory.

**6 - REFERENCES**

S. Irwin: Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiological state of the mouse. *Psychopharmacologia (Berl.)* 13, 222-257 (1968).

**7 - ARCHIVES STATEMENT**

Raw data, protocol and final report were kept in the archives room of ETAP for five years.

**8 - APPENDICES****8.1 - Appendix 1: Study time plan**

**Examination for:** Assessment of behavioral effects of the peptide "ING 913":  
Primary observation in male Wistar rats on the morning.

**Protocole approval:** April, 1999.

**Study period:** April 27 - 30, 1999.

**Draft report date:** September 7, 1999.

**Final report date:** December 7, 1999.

## 8.2 - Appendix 2: Individual results

Treatment	CONTROL			ING 913			ING 913			ING 913		
	Saline			0.5 mg/kg, i.p.			1.5 mg/kg, i.p.			4.5 mg/kg, i.p.		
Time (min.)	30	60	120	30	60	120	30	60	120	30	60	120
Body Position (description)	normal normal normal											
Bizarre Behaviour (description)	normal normal normal											
Exophthalmos (0 or 1)	0 0 0											
Respiratory Rate (1 to 3)	3 3 3	3 2 2	3 3 2	3 3 3	3 3 3	2 2 3	3 3 2	3 3 3	3 3 2	3 3 3	2 3 3	3 3 3
Tremors (0 to 8)	0 0 0											
Twitches (0 to 8)	0 0 0											
Convulsions (0 to 8)	0 0 0											
Transfert Arousal (0 to 8)	3 4 3	3 3 3	3 4 3	3 3 3	3 3 3	2 3 2	3 3 3	3 3 3	3 2 2	4 3 4	3 3 4	4 3 3
Spacial Locomotion (0 to 8)	6 6 2	6 3 1	3 6 1	4 1 2	6 1 4	0 3 1	2 2 4	2 1 2	2 1 1	1 3 3	1 2 1	1 4 1
Palpebral Closure (0 or 1)	0 0 0											
Startle Response (0 to 8)	6 6 4	8 2 2	2 2 0	4 2 6	2 2 4	2 2 6	8 0 4	2 2 2	8 2 2	4 2 0	8 2 2	2 8 2
Piloerection (0 to 8)	0 0 0											
Alley Progression (0 to 8)	4 4 2	6 4 4	6 6 4	4 4 4	4 0 4	4 4 6	6 4 2	6 4 2	6 6 2	2 4 2	4 6 2	4 6 2
Pelvic Elevation (0 to 8)	2 2 1	4 3 1	2 3 2	2 2 2	2 2 2	2 2 2	2 2 1	2 2 1	2 2 2	1 2 1	2 3 1	2 3 2
Tail Elevation (0 to 8)	2 2 2	2 4 2	0 2 2	0 2 2	0 2 2							
Finger Approach (0 to 8)	2 6 0	6 6 6	4 4 0	0 6 4	0 6 6	6 4 0	4 8 6	6 8 4	8 8 0	6 6 2	2 8 0	0 6 6

Treatment	CONTROL			ING 913			ING 913			ING 913		
	Saline			0.5 mg/kg, i.p.			1.5 mg/kg, i.p.			4.5 mg/kg, i.p.		
Time (min.)	30	60	120	30	60	120	30	60	120	30	60	120
Finger Withdrawal (0 to 8)	0	0	0	0	0	0	0	4	2	0	0	0
Touch Escape (0 to 8)	2	4	2	4	0	0	4	4	4	4	4	4
Ataxic Gait (0 to 8)	0	0	0	0	0	0	0	0	0	0	0	0
Hypotonic Gait (0 to 8)	0	0	0	0	0	0	0	0	0	0	0	0
Impaired Gait (0 to 8)	0	0	0	0	0	0	0	0	0	0	0	0
Limb Rotation (0 to 8)	0	0	0	0	0	0	0	0	0	0	0	0
Total Gait Incapacity (0 to 8)	0	0	0	0	0	0	0	0	0	0	0	0
Positional Passivity (0 to 8)	6	6	6	8	8	8	8	2	6	8	8	8
Visual Placing (0 to 8)	8	8	8	8	8	8	8	8	8	8	8	8
Grip Strength (0 to 8)	8	8	8	8	8	8	8	8	8	8	8	8
Body Tone (0 to 8)	6	6	4	6	6	6	6	6	6	6	8	6
Pinna (0 to 8)	0	0	0	0	0	0	0	0	0	0	0	0
Palpebral Reflex (0 or 1)	1	1	1	1	1	1	1	1	1	1	1	1
Toe Pinch (0 to 8)	8	6	8	8	8	8	8	8	8	8	8	8
Position Struggle (0 to 8)	0	0	0	0	1	0	4	2	2	4	3	3
Wire Manoeuvre (0 to 8)	8	6	2	0	0	0	2	2	0	4	4	2
	6	4	4	6	4	4	2	0	0	0	0	0
	0	0	0	2	2	2	2	2	0	4	0	0

Treatment	CONTROL			ING 913			ING 913			ING 913		
	Saline			0.5 mg/kg, i.p.			1.5 mg/kg, i.p.			4.5 mg/kg, i.p.		
Time (min.)	30	60	120	30	60	120	30	60	120	30	60	120
Skin Color (description)	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal
	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal	normal
Erection / Ejaculation (0 or 1)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Diarrhea (0 or 1)	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0
Limb Tone (0 to 8)	6/6	6/6	6/2	8/8	8/8	8/8	4/5	6/6	6/6	8/8	8/8	6/6
	8/8	8/8	8/8	6/6	8/8	8/8	4/4	6/6	6/6	8/8	8/8	8/8
	8/8	4/4	8/8	8/8	8/8	8/8	8/8	8/8	6/6	4/4	6/6	6/6
Abdominal Tone (0 to 8)	6	6	6	6	6	6	6	6	8	6	6	6
	8	6	6	6	8	6	6	6	6	4	6	6
	6	6	6	4	4	4	6	6	6	4	6	6
Pupil Size (0 to 8)	2	2	4	4	6	6	4	4	4	4	6	4
	2	4	2	4	4	4	4	6	4	4	4	6
	6	6	6	4	4	4	2	4	2	4	4	6
Light Pupil Response (0 or 1)	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	1	1
Lacrimation / Salivation (0 or 1)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Provoked Biting (0 to 8)	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0
Tail Pinch (0 to 8)	0	2	6	2	2	2	2	2	0	8	8	8
	4	2	2	3	5	2	6	2	2	2	4	0
	6	6	6	2	0	0	2	3	0	2	2	2
Righting Reflex (0 to 8)	6	7	6	6	8	8	6	7	8	8	8	7
	8	8	8	8	8	8	7	8	8	7	8	8
	8	6	8	7	8	7	7	8	8	8	8	8
Temperature	36.6	36.4	36.9	34.7	35.3	36.6	36.2	36.9	37.4	36.3	34.6	36.4
	36.2	34.3	34.9	36.1	36.8	35.4	36.3	36.5	37.4	37.0	36.2	37.4
	38.9	36.9	35.8	37.1	36.1	36.1	36.2	36.4	35.6	35.9	33.9	35.5
Irritability (0 to 8)	4	3	3	2	3	3	6	2	3	8	8	5
	5	3	3	5	4	4	3	5	4	6	6	7
	4	3	2	5	5	5	3	3	0	5	7	5
Vocalizations (0 to 8)	6	6	6	8	8	6	6	6	4	8	8	8
	8	8	8	6	8	6	8	8	6	4	4	4
	4	2	0	4	4	4	4	4	2	2	6	2
Urination / Defecation	1/5	2/0	1/2	2/5	1/3	0/3	1/8	2/4	0/4	1/5	0/3	1/4
	2/3	1/2	2/1	1/3	1/1	1/2	2/5	3/2	1/5	1/6	2/6	2/2
	2/9	2/2	3/3	1/9	1/3	0/1	1/3	2/3	3/3	1/3	2/4	1/0
Provoked Freezing (0 to 8)	0	2	2	0	0	3	1	0	0	0	3	3
	1	3	0	1	2	2	2	3	2	1	2	2
	0	0	0	0	0	0	0	2	2	2	1	4
Delayed / Acute Death	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

**CONFIDENTIAL**

ETAP CODE N°: INGREDIA 01/0399/ING 913/IRWIN TEST/PM

INGREDIA CODE N°: ING 913/ITPM/R-IP

**ASSESSMENT OF BEHAVIORAL EFFECTS OF "ING 913" PEPTIDE**  
*Primary observation in male Wistar rats on the afternoon*

**FINAL REPORT**

**SPONSOR**

INGREDIA  
51 - 53, Avenue F. Lobbedez B.P. 946  
62033 ARRAS CEDEX - FRANCE

**AUTHORS**

M. MESSAOUDI  
F. DESOR  
D. DESOR

**Representative**

J.-F. BOUDIER  
B. DEMAGNY

Study period: May 4 - 7, 1999

Draft report date: September 7, 1999

Final report date: December 7, 1999

**ETAP - Ethologie Appliquée**

Siège Social : 1, rue Blaise Pascal F-54320 MAXEVILLE - Tél. : 03 83 95 53 00 - Fax : 03 83 98 07 54  
Centre de Recherches : 40, rue Lionnois F-54000 NANCY - Tél. : 03 83 37 26 74 - Fax : 03 83 37 84 77  
E-mail : etap@etap-lab.com

S.A au Capital de 300 000 F - R.C.S. Nancy - Siret : 380 676 767 00011

**CONTENTS**

**QUALITY ASSURANCE STATEMENT** ..... 2

**AUTHORS** ..... 3

**1 - SUMMARY** ..... 4

**2 - INTRODUCTION** ..... 5

**3 - MATERIALS & METHODS** ..... 5

    3.1 - Animals ..... 5

    3.2 - Product ..... 5

    3.3 - Procedure ..... 5

    3.4 - Administration of the product ..... 6

    3.5 - Variables ..... 6

    3.6 - Statistics ..... 6

**4 - RESULTS** ..... 7

    4.1 - Startle response ..... 7

    4.2 - Touch escape ..... 7

    4.3 - Body tone ..... 7

    4.5 - Other tests and safety ..... 7

**5 - CONCLUSION** ..... 10

**6 - REFERENCES** ..... 11

**7 - ARCHIVES STATEMENT** ..... 11

**8 - APPENDICES** ..... 12

    8.1 - Appendix 1: Study time plan ..... 12

    8.2 - Appendix 2: Individual results ..... 13

**QUALITY ASSURANCE STATEMENT**

**STUDY:** ETAP code N°: Ingredia-01/0399/ING 913/Irwin test/PM  
Ingredia code N°: ING 913/ITPM/R-IP

**TITLE:** Assessment of behavioral effects of "ING 913" peptide:  
Primary observation in male Wistar rats on the afternoon.

I, the undersigned, hereby declare that the results presented in this report, in some cases recorded automatically and in others transcribed from the original data sheets, were verified by me item by item in comparison with the original data sheets.

To the best of my knowledge, there were no circumstances that may have affected the quality or integrity of the data.

December 7, 1999

Pr. D. DESOR, Scientific Adviser and Quality Assurance  
Ethopharmacology - Biostatistics

Université Henri Poincaré - Laboratoire de Biologie et Physiologie du Comportement  
U.R.A. C.N.R.S. 1293 - 54500 Vandœuvre-lès-Nancy.

Government authorization to perform experiments on live animals n° 04140/1991.

**AUTHORS**

I, the undersigned, hereby declare that the work described in this report was performed under my supervision as Scientific Director and that the report provides a true and accurate record of the results obtained.

I declare further that the present study was performed in accordance with ETAP's Standard Operating Procedures and in accordance with the principles of Good Laboratory Practice, including appropriate archiving of the original data sheets.

	Signature	Date
M. MESSAOUDI, Scientific Director Biology of Behavior - Ethopharmacology Government authorization to perform experiments on live animals n° 04535/1991		December 7, 1999

We the undersigned, responsible for the execution of the experiments described in this report hereby, declare that the experiments were performed as described and that the data presented correspond exactly to the results obtained during the experiments.

	Signature	Date
F. DESOR, Research Technician Ethopharmacology Authorization to perform experiments on live animals		December 7, 1999

The present study was carried out in ETAP's Research Center  
40, rue Lionnois F-54000 Nancy - Government authorization n° B54805

## 1 - SUMMARY

Behavioral effects of ING 913 peptide were assessed on the afternoon using Irwin's test in male Wistar rats. The drug was administered at the doses of 0.5, 1.5 and 4.5 mg/kg, i.p., 30 minutes before the first observation. Control rats received saline solution and were tested in the same conditions.

In the following study, the characteristics of ING 913 that appear are in favor of an antistress profile which is apparent from:

- decreased reaction to startle at 0.5 and 1.5 mg/kg;
- decreased reaction to touch escape at 0.5, 1.5 and 4.5 mg/kg.

At the doses of 0.5 and 4.5 mg/kg, ING 913 induced body tone (i.e. resistance to compression between two fingers) at 120 minutes after the treatment administration.

The safety profile is satisfactory.

## 2 - INTRODUCTION

ETAP-Ethologie Appliquée was asked by INGREDIA S.A. to investigate the behavioral effects of the peptide "ING 913", administered on the afternoon at the doses of 0.5, 1.5 and 4.5 mg/kg, i.p., dissolved in 0.9% NaCl solution, using a study method derived from that of Irwin (1968) in male Wistar rats.

## 3 - MATERIALS & METHODS

### 3.1 - Animals

Eleven male Wistar rats (Centre d'élevage Iffa Credo, L'Arbresle - France), weighing 260-300 g at the start of experiment, were used for the study. They were housed in groups of two or three in a climate controlled room with a 12-h light:dark cycle (light off at 8 a.m.) with access to food and water *ad libitum*.

After seven day acclimatization period, following their entry in laboratory, the rats were weighed, identified and then randomly assigned to one of the four treatment groups (n = 2 or 3).

### 3.2 - Product

**Table 1**  
**Product**

Product	ING 913	Saline
Origin	Génosphère France	B.Braun Fandre France
Batch number	6986	426350
Expiry date	-	12/2003
Preparation	Diluted in 0.9% NaCl solution	-
Storage	At -20° C. protected from the light	At 4°C. protected from the light

### 3.3 - Procedure

The rats were injected at 2 p.m. with the "ING 913" peptide solutions and then observed in comparison with control ones given vehicle. Observations were performed 30, 60 and 120 minutes after administration of the peptide and also 24 hours later for delayed death.

### 3.4 - Administration of the product

The product "ING 913" was dissolved in 0.9% NaCl solution on a magnetic stirrer for 30 minutes. The solutions were prepared just before their i.p. administration, 30 minutes prior to the primary observation test (Tab. 2).

**Table 2**  
**Administration of the product**

Group	Rats per group	Treatment	Dose (mg/kg, i.p.)	Volume ml/kg	Administration before test (minutes)
Control	3	Saline	-	2	30
Group 1	3	ING 913	0.5	2	30
Group 2	3	ING 913	1.5	2	30
Group 3	2	ING 913	4.5	2	30

### 3.5 - Variables

The following items concerning behavioral changes, but also physiological and toxic symptoms and mortality were recorded:

- Behavior:

- spontaneous activity;
- motor affective response;
- sensorimotor response.

- Neurology:

- posture;
- muscle tone;
- equilibrium and gait;
- CNS excitement.

- Autonomic NS:

- eyes;
- secretions and excretions;
- miscellaneous.

- Toxicology:

- mortality.

### 3.6 - Statistics

Only for information, Mann-Whitney U-test was used to compare ING 913-treated rats to control ones.

## 4 - RESULTS

Tables 3a and 3b showed all the assessed behavioral effects of the peptide "ING 913", observed at 30, 60 and 120 minutes after its administration at the doses of 0.5, 1.5 and 4.5 mg/kg, i.p., in male Wistar rats.

### 4.1 - Startle response

This is defined as a sudden body jerking movement of the animal in response to a noisy tool snap. It is increased by fearfulness or increasing CNS excitability.

ING 913-treated rats, at the doses of 0.5 and 1.5 mg/kg, showed less reaction than control ones at 30 minutes after the treatment administration.

### 4.2 - Touch escape

This is defined as response of the animal, when it is grasped. It is scored in terms of the vigor of the escape.

ING 913-treated rats, at the doses of 0.5, 1.5 and 4.5 mg/kg, showed less reaction than control ones at 30 minutes after the treatment administration.

### 4.3 - Body tone

This variable is determined by compressing the sides of the animal between the lower thorax and pelvis several times at approximately one second intervals, using the thumb and index finger.

ING 913-treated rats, at the doses of 0.5 and 4.5 mg/kg, showed more body tone than control ones at 120 minutes after the treatment administration.

### 4.4 - Other tests and safety

Tables 3a and 3b list all the tests performed. Only those where treated animals showed modified responses have been detailed above.

In particular there were no observed anomalies of skin color. There was no bizarre behavior, no tremors, no twitching, no convulsions, no irritability and no episodes of ataxia. There was no diarrhea, no effect on pupil diameter and no exophthalmos.

From the safety point of view no animal died during observation and all were sacrificed after 24 hours.

**Table 3a**  
**Behavioral effects of the peptide "ING 913"**

<b>Time</b>	<b>30 minutes</b>	<b>60 minutes</b>	<b>120 minutes</b>
Body Position	N.S.	N.S.	N.S.
Bizarre Behaviour	None	None	None
Exophthalmos	None	None	None
Respiratory Rate	N.S.	N.S.	N.S.
Tremors	None	None	None
Twitches	None	None	None
Convulsions	None	None	None
Transfer Arousal	N.S.	N.S.	N.S.
Spatial Locomotion	N.S.	N.S.	N.S.
Palpebral Closure	N.S.	N.S.	N.S.
Startle Response	<b>* Less reaction at 0.5 mg/kg 1.5 mg/kg</b>	N.S.	N.S.
Piloerection	None	None	None
Alley Progression	N.S.	N.S.	N.S.
Pelvic Elevation	N.S.	N.S.	N.S.
Tail Elevation	N.S.	N.S.	N.S.
Finger Approach	N.S.	N.S.	N.S.
Finger Withdrawal	N.S.	N.S.	N.S.
Touch Escape	<b>* Less reaction at 0.5 mg/kg 1.5 mg/kg 4.5 mg/kg</b>	N.S.	N.S.
Ataxic Gait	N.S.	N.S.	N.S.
Hypotonic Gait	N.S.	N.S.	N.S.
Impaired Gait	N.S.	N.S.	N.S.
Limb Rotation	N.S.	N.S.	N.S.
Total Gait Incapacity	N.S.	N.S.	N.S.
Positional Passivity	N.S.	N.S.	N.S.

\*  $p < 0.05$ ; N.S., non significant (Mann-Whitney U-test: ING 913 vs. Control).

**Table 3b**  
**Behavioral effects of the peptide "ING 913"**

Time	30 minutes	60 minutes	120 minutes
Visual Placing	N.S.	N.S.	N.S.
Grip Strength	N.S.	N.S.	N.S.
Body Tone	N.S.	N.S.	<b>*More tone at 0.5 mg/kg 4.5 mg/kg</b>
Pinna	N.S.	N.S.	N.S.
Palpebral Reflex	N.S.	N.S.	N.S.
Toe Pinch	N.S.	N.S.	N.S.
Position Struggle	N.S.	N.S.	N.S.
Wire Manoeuvre	N.S.	N.S.	N.S.
Skin Color	N.S.	N.S.	N.S.
Erection / Ejaculation	None	None	None
Diarrhea	None	None	None
Limb Tone	N.S.	N.S.	N.S.
Abdominal Tone	N.S.	N.S.	N.S.
Pupil Size	N.S.	N.S.	N.S.
Light Pupil Response	N.S.	N.S.	N.S.
Lacrimation / Salivation	None	None	None
Provoked Biting	None	None	None
Tail Pinch	N.S.	N.S.	N.S.
Righting Reflex	N.S.	N.S.	N.S.
Temperature	N.S.	N.S.	N.S.
Irritability	N.S.	N.S.	N.S.
Vocalizations	N.S.	N.S.	N.S.
Urination / Defecation	N.S.	N.S.	N.S.
Provoked Freezing	N.S.	N.S.	N.S.
Delayed / Acute Death	None	None	None

\*  $p < 0.05$ ; N.S., non significant (Mann-Whitney U-test: ING 913 vs. Control).

## 5 - CONCLUSION

Behavioral effects of ING 913 were assessed on the afternoon using Irwin's test in male W rats. The drug was administered at the doses of 0.5, 1.5 and 4.5 mg/kg, i.p., 30 minutes before the first observation. Control rats received saline solution and were tested in the same conditions.

In our experimental conditions, the characteristics of ING 913 that appear are in favor of an antistress profile which is apparent from decreased reaction to startle at 0.5 and 1.5 mg/kg and touch escape at 0.5, 1.5 and 4.5 mg/kg.

At the doses of 0.5 and 4.5 mg/kg, ING 913 induced body tone (i.e. resistance to compression between two fingers) at 120 minutes after the treatment administration.

The safety profile is satisfactory.

## 6 - REFERENCES

S. Irwin: Comprehensive observational assessment: Ia. A systematic, quantitative procedure assessing the behavioral and physiological state of the mouse. *Psychopharmacologia (Berl)* 13, 222-257 (1968).

## 7 - ARCHIVES STATEMENT

Raw data, protocol and final report were kept in the archives room of ETAP for five years.

**8 - APPENDICES****8.1 - Appendix 1: Study time plan**

**Examination for:** Assessment of behavioral effects of the peptide "ING 913":  
Primary observation in male Wistar rats on the afternoon.

**Protocole approval:** April, 1999.

**Study period:** May 4 - 7, 1999.

**Draft report date:** September 7, 1999.

**Final report date:** December 7, 1999.

8.2 - Appendix 2: Individual results

Treatment	CONTROL			ING 913			ING 913			ING 913		
	Saline			0.5 mg/kg, i.p.			1.5 mg/kg, i.p.			4.5 mg/kg, i.p.		
Time (min.)	30	60	120	30	60	120	30	60	120	30	60	120
Body Position (description)	normal normal normal	normal normal -	normal normal -	norm norm -								
Bizarre Behaviour (description)	normal normal normal	normal normal -	normal normal -	norm norm -								
Exopthalmos (0 or 1)	0 0 0	0 0 -	0 0 -	0 0 -								
Respiratory Rate (1 to 3)	3 3 3	2 3 3	3 3 3	3 3 3	2 2 3	3 2 3	2 3 3	2 3 3	2 3 2	2 2 -	3 2 -	3 2 -
Tremors (0 to 8)	0 0 0	0 0 -	0 0 -	0 0 -								
Twitches (0 to 8)	0 0 0	0 0 -	0 0 -	0 0 -								
Convulsions (0 to 8)	0 0 0	0 0 -	0 0 -	0 0 -								
Transfert Arousal (0 to 8)	3 3 4	3 4 4	3 4 3	3 3 4	3 3 4	3 3 4	4 3 4	3 3 4	3 3 3	4 4 -	4 4 -	4 4 -
Spacial Locomotion (0 to 8)	4 3 2	3 2 4	1 3 1	2 0 3	1 0 4	4 0 3	4 1 4	1 1 2	1 1 3	3 4 -	3 2 -	4 1 -
Palpebral Closure (0 or 1)	0 0 0	0 0 -	0 0 -	0 0 -								
Startle Response (0 to 8)	6 6 4	2 6 6	2 2 2	2 2 4	2 4 2	2 2 0	2 4 2	2 4 2	2 2 0	4 4 -	4 4 -	2 4 -
Piloerection (0 to 8)	0 0 0	0 0 -	0 0 -	0 0 -								
Alley Progression (0 to 8)	2 2 2	0 2 4	0 2 2	2 2 2	2 4 2	4 4 4	4 4 2	4 2 2	4 2 2	4 2 -	4 4 -	4 4 -
Pelvic Elevation (0 to 8)	2 1 0	1 1 1	1 1 1	1 1 1	1 2 1	1 2 1	2 2 2	2 1 2	2 1 2	2 1 -	1 2 -	1 2 -
Tail Elevation (0 to 8)	2 0 2	2 0 2	0 2 2	2 2 2	2 2 2	2 2 2	2 2 1	2 2 0	2 0 0	2 2 -	2 2 -	2 2 -
Finger Approach (0 to 8)	6 6 8	0 4 0	0 4 0	6 6 8	8 0 6	6 0 6	0 0 6	0 0 8	6 0 6	8 6 -	4 6 -	4 0 -

Treatment	CONTROL			ING 913			ING 913			ING 913	
	Saline			0.5 mg/kg, i.p.			1.5 mg/kg, i.p.			4.5 mg/kg, i.p.	
Time (min.)	30	60	120	30	60	120	30	60	120	30	60
Finger Withdrawal (0 to 8)	0	0	0	0	0	0	0	0	0	0	0
Touch Escape (0 to 8)	4	2	2	2	0	0	2	0	0	0	0
Ataxic Gait (0 to 8)	4	2	0	2	2	0	2	2	0	0	2
Hypotonic Gait (0 to 8)	4	0	0	0	1	0	0	0	0	-	-
Impaired Gait (0 to 8)	0	0	0	0	0	0	0	0	0	0	0
Limb Rotation (0 to 8)	0	0	0	0	0	0	0	0	0	0	0
Total Gait Incapacity (0 to 8)	0	0	0	0	0	0	0	0	0	0	0
Positional Passivity (0 to 8)	0	0	0	0	0	0	0	0	0	-	-
Visual Placing (0 to 8)	8	8	8	4	8	8	6	6	6	8	6
Grip Strength (0 to 8)	6	8	8	8	6	8	8	8	8	8	6
Body Tone (0 to 8)	8	8	8	8	8	8	6	6	6	-	-
Pinna (0 to 8)	8	8	8	8	8	8	8	8	8	8	8
Palpebral Reflex (0 or 1)	8	8	8	8	8	8	8	8	8	8	8
Toe Pinch (0 to 8)	8	8	8	8	8	8	8	8	8	-	-
Position Struggle (0 to 8)	4	6	6	6	6	6	4	4	4	4	4
Wire Manoeuvre (0 to 8)	2	3	4	4	6	6	6	6	6	6	4
	6	6	4	6	6	6	4	4	4	-	-
Pinna (0 to 8)	0	0	0	0	0	0	0	0	0	0	0
Palpebral Reflex (0 or 1)	0	0	0	0	0	0	0	0	0	0	0
Toe Pinch (0 to 8)	1	1	1	1	1	1	1	1	1	1	1
Position Struggle (0 to 8)	1	1	1	1	1	1	1	1	1	1	1
Wire Manoeuvre (0 to 8)	1	1	1	1	1	1	1	1	1	-	-
Toe Pinch (0 to 8)	4	8	6	8	8	8	6	8	8	8	8
Position Struggle (0 to 8)	6	8	8	8	8	6	8	8	8	8	8
Wire Manoeuvre (0 to 8)	8	8	8	8	8	8	8	8	8	-	-
Position Struggle (0 to 8)	4	5	0	0	1	2	2	1	0	0	0
Wire Manoeuvre (0 to 8)	1	1	1	0	0	0	1	0	1	1	1
Wire Manoeuvre (0 to 8)	0	2	1	1	1	0	1	2	0	-	-
Wire Manoeuvre (0 to 8)	8	8	4	4	4	2	2	0	0	2	2
Wire Manoeuvre (0 to 8)	4	0	0	2	4	0	4	2	0	4	0
Wire Manoeuvre (0 to 8)	4	6	4	6	6	0	2	0	0	-	-

Treatment	CONTROL			ING 913			ING 913			ING 913		
	Saline			0.5 mg/kg, i.p.			1.5 mg/kg, i.p.			4.5 mg/kg, i.p.		
Time (min.)	30	60	120	30	60	120	30	60	120	30	60	120
Skin Color (description)	normal normal normal											
Erection / Ejaculation (0 or 1)	0/0 0/0 0/0											
Diarrhea (0 or 1)	0 0 0											
Limb Tone (0 to 8)	6/6 6/6 6/6	6/6 6/6 6/6	6/6 6/6 6/6	6/6 6/4 6/6	6/6 5/5 6/6	6/6 6/6 6/6	6/6 6/6 6/6	4/6 6/6 6/6	6/6 6/6 6/6	6/6 6/6 6/6	6/6 6/6 6/6	6/6 6/6 6/6
Abdominal Tone (0 to 8)	6 6 4	6 6 6	6 6 4	6 4 6	6 4 6	6 6 6	6 6 6	6 6 6	6 4 6	8 6 -	6 6 -	6 6 -
Pupil Size (0 to 8)	4 4 4	4 4 4	2 4 4	4 4 2	4 4 4	4 4 4	4 4 4	4 4 2	4 4 2	4 4 -	4 4 -	4 4 -
Light Pupil Response (0 or 1)	1 1 1	1 1 -	1 1 -	1 1 -								
Lacrimation / Salivation (0 or 1)	0/0 0/0 0/0											
Provoked Biting (0 to 8)	0 0 0											
Tail Pinch (0 to 8)	2 8 8	0 0 8	0 8 2	2 4 5	8 2 8	2 0 0	8 8 8	3 8 8	8 8 8	8 4 -	8 4 -	8 8 -
Righting Reflex (0 to 8)	8 8 8	8 8 8	7 8 8	8 8 8	8 8 8	8 8 8	8 8 8	8 8 8	8 8 8	8 8 -	8 8 -	8 8 -
Temperature	35.8 34.7 36.2	35.9 37.9 33.9	35.0 34.2 34.9	35.3 36.4 34.9	36.1 34.7 34.9	34.6 34.5 37.5	34.3 36.5 36.7	34.7 37.7 37.6	37.7 36.2 34.8	35.7 37.9 -	36.6 35.2 -	34.4 36.6 -
Irritability (0 to 8)	5 4 3	6 2 3	3 2 2	1 4 3	1 3 3	2 1 3	4 5 4	3 3 3	4 3 3	1 2 -	2 3 -	3 4 -
Vocalizations (0 to 8)	2 1 6	2 1 6	1 0 6	2 2 7	2 2 6	2 2 5	4 2 4	4 6 5	2 2 5	2 5 -	2 4 -	2 6 -
Urination / Defecation	1/6 2/3 1/2	2/0 1/3 2/1	1/3 1/0 3/3	2/4 2/6 1/5	0/9 1/0 1/2	3/4 2/3 1/0	1/7 2/8 1/5	1/6 1/2 3/1	1/3 1/3 1/0	2/10 2/5 -	1/2 1/2 -	1/2 2/0 -
Provoked Freezing (0 to 8)	0 0 2	0 0 2	1 0 2	0 0 0	1 0 0	1 2 2	0 0 2	1 0 2	1 2 1	1 2 -	2 0 -	2 2 -
Delayed / Acute Death	0/0 0/0 0/0											