

**CONFIDENTIAL**



**ETAP CODE N°: INGRECIA 3/0298/ PN-EPC**  
**INGREDIA CODE N°: ING 912/CPFR/R-IP**

**CONFIDENTIEL**

**ADDICTIVE EFFECT OF THE PRODUCT "ING 912"  
INTRAPERITONEALLY ADMINISTERED  
IN THE CONDITIONED PLACE PREFERENCE  
IN THE MALE WISTAR RAT**

**FINAL REPORT**

**ADDRESSEE**

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*Study Period: April - May, 1998.*

*Final Report Date: October 26, 1998*

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**QUALITY ASSURANCE STATEMENT**

STUDY: ETAP CODE N°:           INGREDIA 3/0298/PN-EPC  
          INGREDIA CODE N°:       ING 912/CPPR/R-IP

TITLE:   Addictive effect of the product "ING 912", intraperitoneally administered, in the Conditioned Place Preference test in the male Wistar rat.

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.

October 26, 1998

Pr. D. DESOR, Scientific Adviser and Quality Assurance  
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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 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.

I declare further that the rats used in the present study were treated according to the rules provided by the ASAB Ethical Committee (Guidelines for the use of animals in research; *Animal Behavior*, 45: 209-212, 1993).

October 26, 1998

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



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.

October 26, 1998

P.-H. JUNG, Study Director  
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The present study was carried out in ETAP's Research Center - 40, rue Lionnois  
F-54000 Nancy - Government authorization n° A54805.

**1 - SUMMARY**

***ADDICTIVE EFFECT OF THE PRODUCT "ING 912"  
INTRAPERITONEALLY ADMINISTERED IN THE CONDITIONED PLACE  
PREFERENCE IN THE MALE WISTAR RAT***

The product "ING 912" from INGREDIA, was tested for its possible addictive effect using the paradigm of Conditioned Place Preference in the male Wistar rat.

It was studied at a dose of 1 mg/kg dissolved in 0.9% NaCl solution and i.p. administered on days 4, 6, 8 and 10. On alternate days, each rat received a vehicle injection.

Diazepam (3 mg/kg, i.p.) was used as a reference substance and administered according to the same design plan.

Control rats were treated with saline during the same period.

Conditioned place preference was expressed as a positive difference in time spent on the unconditioned stimulus associated side between post and preconditioning tests.

Control rats and those treated with the product "ING 912" spent as much time in the non-preferred compartment on both sessions I and III and tended to increase their number of crossings between the two compartments on session III compared to session I.

Diazepam-treated rats significantly increased time spent in the non-preferred compartment and their number of crossings between the two compartments on session III compared with session I.

Unlike diazepam (3 mg/kg, i.p.), the product "ING 912" (1 mg/kg, i.p.), did not show any addictive effect, since it did not induce conditioned place preference in the male Wistar rat.

**2 - INTRODUCTION**

ETAP-Ethologie Appliquée was asked by INGREDIA to investigate the possible addictive effect using the paradigm of Conditioned Place Preference in the male Wistar rat.

It was studied at a dose of 1 mg/kg dissolved in 0.9% NaCl solution and i.p. administered on days 4, 6, 8 and 10. On alternate days, each rat received a vehicle injection.

Diazepam (3 mg/kg, i.p.) was used as a reference substance and administered according to the same design plan.

Control rats were treated with saline during the same period.

Conditioned place preference was expressed as a positive difference in time spent on the unconditioned stimulus associated side between post and preconditioning tests.

### **3 - MATERIALS AND METHODS**

#### **3.1 - Conditioned Place Preference test**

In the Conditioned Place Preference test, the rat associates an "internal state of well-being" with a distinctive environment, the subsequent selection of which is considered to reflect the appetitive properties of the drug. After a certain number of administrations of an addicting compound, followed by a forced stay in the compartment initially non-preferred, the addicted animal will preferentially choose this compartment.

Opiates, such as morphine (1), psychostimulants, such as amphetamine (2) and minor tranquilizers, such as diazepam (3, 4) have been shown to induce conditioned place preference.

#### **3.2 - Animals**

Forty male Wistar rats AF EOPS (Centre d'élevage Iffa-Credo, 69 - St-Germain sur l'Arbresle, France), weighing 280 to 300 g were used. On receipt the rats were identified and then housed in polycarbonate cages 48 x 27 x 20 cm (U.A.R., 91 - Epinay-Sur-Orge, France) in an air-conditioned room maintained at a relatively constant temperature ( $22 \pm 2^\circ\text{C}$ ) and with a 12 hour light-dark cycle. Tap water and standard diet (food pellets M25, Ets Piétrement, 77 - Provins, France) were available *ad libitum*.

After an acclimatization period of one week after the day of arrival, the rats were weighed and randomly put into 3 groups (n = 12 to 16). The rats of various groups were all handled in the same way and under the same conditions.

#### **3.3 - Material**

The experimental apparatus consists in a rectangular box (50 x 25 x 40 cm) divided into two compartments separated by a guillotine door. The compartments are characterized by the colour of their walls and the texture of their floors: black walls with smooth floor vs. grey walls with corrugated floor.

**3.4 - Products (Tab. 1)****Table 1**  
**Products**

<b>Products</b>	<b>Saline</b>	<b>Diazepam</b>	<b>ING 912</b>
<b>Origin</b>	B. Braun Fandre France	Produits Roche France	INGREDIA France
<b>Batch number</b>	62215	323824	Not communicated
<b>Storage</b>	At 4°C. protected from the light	At 4°C. protected from the light	At 4°C. protected from the light

**3.5 - Procedure**

The test was performed and the recorded behaviors were scored by experimenters unaware of the administered products.

Session I: This session was carried out over 3 days, 30 minutes per day, in order to familiarize the rats with the apparatus. The animal was allowed to move freely between compartments. On the third day each rat's preference for one of the two compartments was determined (compartment where the rat spent the more time).

Session II: On days 4, 6, 8 and 10, rats were treated with the tested compound and individually enclosed into their initially non-preferred compartment for 45 minutes.

On alternate days, each rat received a vehicle injection and was individually enclosed into the initially preferred compartment for 45 minutes.

Session III: On the 12th day, the addiction test was carried out: the rats were individually placed between the compartments for 30 minutes with free access to both compartments. The time spent in each compartment was measured.

Sessions I and III were monitored and recorded on VHS-videotapes.

### 3.6 - Administration of products (Tab. 2)

The treatments were randomly pre-attributed by a computer system in each series of three animals.

The product "ING 912" and diazepam were dissolved or suspended in 0.9% NaCl solution on a magnetic stirrer for 30 minutes. Before each session the rats received an i.p. injection of either the test product or vehicle. The test product was always associated with the non-preferred compartment.

**Table 2**  
Administration of products

Products	N	Products injected Days: 4, 6, 8 and 10	Vehicle injected Days: 5, 7, 9 and 11	Volume (ml/kg)	Administration before the addiction test Day: 12
Saline	16	Saline	Saline	2	30 minutes
Diazepam	12	Diazepam (3 mg/kg, i.p.)	Saline	2	30 minutes
ING 912	12	ING 912 (1 mg/kg, i.p.)	Saline	2	30 minutes

### 3.7 - Variables

- time spent in each compartment and the number of crossings on the 3rd day of session I (determination of the initially preferred compartment);
- time spent in each compartment and the number of crossings during session III (determination of addiction).

### 3.8 - Statistics

For each group, a paired t-test (2-tailed) was used to compare the time spent in the non-preferred compartment and the number of crossings on the 3rd day of session I with the time spent in the non-preferred compartment and the number of crossings on session III. Data were reported as mean  $\pm$  s.e.m. (standard error of the mean). Differences were considered to be significant at  $p < 0.05$ .

All the statistical analyses were carried out using the Statview<sup>®</sup> 4.1 package.

## 4 - RESULTS

**Comment:** The results of the present study concerned 39 rats. One saline rat exhibited freezing behavior during session I; therefore it was discarded from the statistical analysis.

### 4.1 - Time spent in the non-preferred compartment (Tab. 3 & Fig. 1)

Control rats and those treated with the product "ING 912" spent as much time in the non-preferred compartment on both sessions I and III.

Diazepam-treated rats significantly increased time spent in the non-preferred compartment on session III compared with session I.

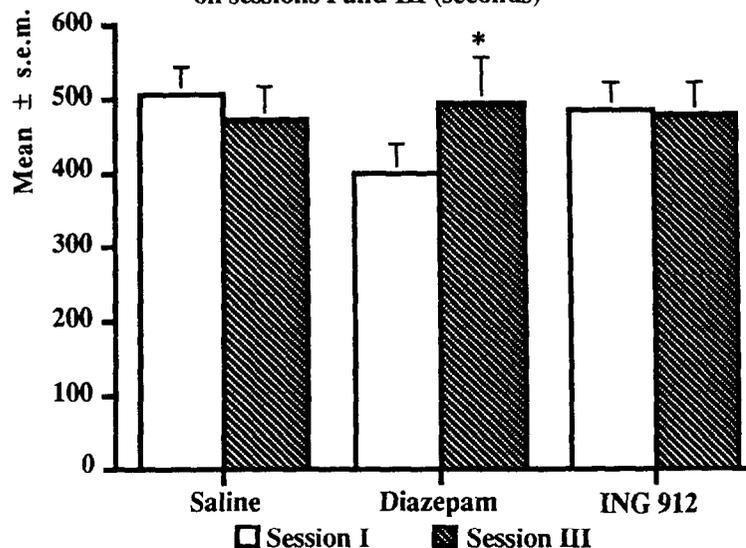
**Table 3**

Effects of products on time spent in the non-preferred compartment on sessions I and III (seconds)

Products	Saline		Diazepam		ING 912	
Rats per group	15		12		12	
Dose (mg/kg, i.p.)	-		3		1	
	Session I	Session III	Session I	Session III	Session I	Session III
Mean ± s.e.m.	508.13 ± 34.75	473.07 ± 42.41	403.42 ± 37.39	497.17 ± 59.16	486.92 ± 36.30	481.33 ± 40.37
Paired t-test	t = 0.78		t = 2.28		t = 0.11	
Significance	N.S.		p < 0.05		N.S.	

**Figure 1**

Effects of products on time spent in the non-preferred compartment on sessions I and III (seconds)



Paired t-test: \* p < 0.05

#### 4.2 - Number of crossings between the two compartments (Tab. 4 & Fig. 2)

Control rats and those treated with the product "ING 912" tended to increase their number of crossings between the two compartments on session III compared to session I. However the differences were not significant.

Diazepam-treated rats significantly increased their number of crossings between the two compartments on session III compared with session I.

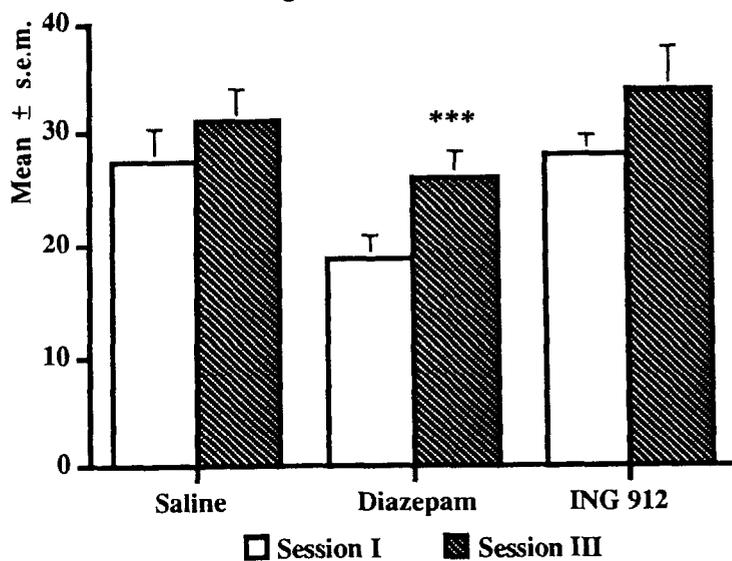
**Table 4**

Effects of products on the number of crossings between the two compartments during sessions I and III

Products	Saline		Diazepam		ING 912	
Rats per group	15		12		12	
Dose (mg/kg, i.p.)	-		3		1	
	Session I	Session III	Session I	Session III	Session I	Session III
Mean ± s.e.m.	27.73 ± 2.72	31.33 ± 2.82	18.92 ± 2.17	26.33 ± 2.17	28.25 ± 1.62	34.17 ± 3.81
Paired t-test	t = 2.05		t = 4.58		t = 1.72	
Significance	N.S.		p < 0.005		N.S.	

**Figure 2**

Effects of products on the number of crossings between the two compartments during sessions I and III



Paired t-test: \*\*\* p < 0.005

**5 - CONCLUSION**

The product "ING 912" from INGREDIA, was tested in blind conditions for its possible addictive effect using the paradigm of Conditioned Place Preference in the male Wistar rat.

It was studied at a dose of 1 mg/kg dissolved in 0.9% NaCl solution and i.p. administered on days 4, 6, 8 and 10. On alternate days, each rat received a vehicle injection.

Diazepam (3 mg/kg, i.p.) was used as a reference substance and administered according to the same design plan.

Control rats were treated with saline during the same period.

Conditioned place preference was expressed as a positive difference in time spent on the unconditioned stimulus associated side between post and preconditioning tests.

Control rats and those treated with the product "ING 912" spent as much time in the non-preferred compartment on both sessions I and III and tended to increase their number of crossings between the two compartments on session III compared to session I.

Diazepam-treated rats significantly increased time spent in the non-preferred compartment and their number of crossings between the two compartments on session III compared to session I.

**In our experimental conditions, unlike diazepam (3 mg/kg, i.p.), the product "ING 912" (1 mg/kg, i.p.), did not show any addictive effect, since it did not induce conditioned place preference in the male Wistar rat.**

**6 - ARCHIVES STATEMENT**

Raw data, protocol and final report were kept in the archives room of ETAP for five years and VHS-videotapes for one year.

**7 - REFERENCES**

- 1 - Mucha, R. F., Van der Kooy, D. O'Shaughnessy, M. and Buceniaks, P. (1982): Drug reinforcement studied by the use of place contioning in rat. *Brain Res.* 243: 91-105.
- 2 - Reicher, M. A., and Holman, E. W. (1977): Location preference and flavor aversion reiforced by amphetamine in rats. *Anim. Learn. Behav.* 5: 343-346.
- 3 - File, S: E. (1986): Aversive and appetitive properties of anxiogenic and anxiolytic agents. *Behav. Brain Res.* 21: 189-194.
- 4 - Spiraki, C., Kazandjian, A. and Varonos, D. (1985): Diazepam induced place preference conditioning: appetitive and anti-aversive properties. *Psychopharmacology.* 87: 225-232.

**8 - APPENDICES****8.1 - Individual results**

Products	SESSION I		SESSION III	
	Time spent in the non-preferred compartment (s)	Number of crossings	Time spent in the non-preferred compartment (s)	Number of crossings
Saline	448	50	518	49
Saline	469	27	168	14
Saline	609	47	491	47
Saline	435	21	211	19
Saline	484	13	603	26
Saline	402	25	462	28
Saline	359	21	585	25
Saline	642	25	281	23
Saline	696	38	568	41
Saline	372	27	529	36
Saline	410	35	533	49
Saline	411	20	476	29
Saline	831	22	827	29
Saline	488	17	400	23
Saline	566	28	444	32
Saline	Discarded (freezing)			
Diazepam	262	16	205	23
Diazepam	310	22	293	27
Diazepam	503	27	576	31
Diazepam	427	24	409	34
Diazepam	413	24	375	21
Diazepam	295	20	547	30
Diazepam	388	9	497	17
Diazepam	466	10	620	23
Diazepam	722	31	850	39
Diazepam	465	23	782	30
Diazepam	279	10	588	29
Diazepam	311	11	224	12
ING 912	317	25	378	33
ING 912	612	38	349	24
ING 912	608	34	519	66
ING 912	350	17	721	20
ING 912	333	32	438	44
ING 912	439	31	519	39
ING 912	702	31	473	35
ING 912	484	31	632	32
ING 912	523	24	371	30
ING 912	608	26	655	41
ING 912	401	26	237	14
ING 912	466	24	484	32

## 8.2 - Study proposal

1

### STUDY PROPOSAL N° INGREDIA 3/0298/PN-EPC

#### *EFFECT OF THE PRODUCT "ING 912" INTRAPERITONEALLY ADMINISTERED ON THE CONDITIONED PLACE PREFERENCE IN THE MALE WISTAR RAT*

##### INTRODUCTION

ETAP-Ethologie Appliquée is asked by INGREDIA to investigate the possible addictive effect of the product "ING 912" using the paradigm of Conditioned Place Preference in the male Wistar rat.

The product "ING 912" from INGREDIA, was tested for its possible addictive effect using the paradigm of Conditioned Place Preference in the male Wistar rat.

It was studied at a dose of 1 mg/kg dissolved in 0.9% NaCl solution and i.p. administered on days 4, 6, 8 and 10. On alternate days, each rat received a vehicle injection.

Diazepam (3 mg/kg, i.p.) was used as a reference substance and administered according to the same design plan.

Control rats were treated with saline during the same period.

Conditioned place preference was expressed as a positive difference in time spent on the unconditioned stimulus associated side between post and preconditioning tests.

##### MATERIALS AND METHODS

###### Conditioned Place Preference test

In the Conditioned Place Preference test, the rat associates an "internal state of well-being" with a distinctive environment, the subsequent selection of which is considered to reflect the appetitive properties of the drug. After a certain number of administrations of an addicting compound, followed by a forced stay in the compartment initially non-preferred, the addicted animal will preferentially choose this compartment.

Opiates, such as morphine, psychostimulants, such as amphetamine and minor tranquilizers, such as diazepam have been shown to induce conditioned place preference.

###### Animals

Forty male Wistar rats AF EOPS (Centre d'élevage Iffa-Credo, 69 - St-Germain sur l'Arbresle, France), weighing 280 to 300 g are used. On receipt the rats are identified and then housed in polycarbonate cages 48 x 27 x 20 cm (U.A.R., 91 - Epinay-Sur-Orge, France) in an air-conditioned room maintained at a relatively constant temperature (22 ± 2°C) and with a 12 hour light-dark cycle. Tap water and standard diet (food pellets M25, Ets Piétrement, 77 - Provins, France) are available *ad libitum*.

After an acclimatization period of 7 days after the day of arrival, the rats are weighed and randomly put into 3 groups. The rats of various groups are all handled in the same way and under the same conditions.

### **Material**

The experimental apparatus consists in a rectangular box (50 x 25 x 40 cm) divided into two compartments separated by a guillotine door. The compartments are characterized by the colour of their walls and the texture of their floors: black walls with smooth floor vs. grey walls with corrugated floor.

### **Procedure**

The test is performed and the recorded behaviors are scored by experimenters unaware of the administered products.

**Session I:** This session is carried out over 3 days, 15 minutes per day, in order to familiarize the rats with the apparatus. The animal is allowed to move freely between compartments. On the third day each rat's preference for one of the two compartments is determined (compartment where the rat spends the more time).

**Session II:** On days 4, 6, 8 and 10, rats are treated with the tested product and individually enclosed into their initially non-preferred compartment for 45 minutes.

On alternate days, each rat receives a vehicle injection and is individually enclosed into the initially preferred compartment for 45 minutes.

**Session III:** On the 12th day, the addiction test is carried out: the rats are individually placed between the compartments for 15 minutes with free access to both compartments. The time spent in each compartment is measured.

Sessions I and III are monitored and recorded on VHS-videotapes.

### **Administration of products**

The treatments are randomly pre-attributed by a computer system in each series of 3 rats. The product "ING 912" and diazepam are dissolved or suspended in 0.9% NaCl solution on a magnetic stirrer for 30 minutes. Before each session the rats receive an i.p. injection of either the test product or vehicle. Test product is always associated with the non-preferred compartment.

**Table 1**  
Administration of products

Products	N	Products injected Days :4, 6, 8 and 10	Vehicle injected Days : 5, 7, 9 and 11	Volume (ml/kg)	Administration before the addiction test Day: 12
Saline	16	Saline	Saline	2	30 minutes
Diazepam	12	Diazepam (3 mg/kg, i.p.)	Saline	2	30 minutes
ING 912	12	ING 912 (1 mg/kg, i.p.)	Saline	2	30 minutes

### **Variables**

- time spent in each compartment and the number of crossings on the 3rd day of session I (determination of the initially preferred compartment);
- time spent in each compartment and the number of crossings during session III (determination of addiction).

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**Statistics**

For each group, a paired t-test (2-tailed) is used to compare the time spent in the non-preferred compartment and the number of crossings on the 3rd day of session I with the time spent in the non-preferred compartment and the number of crossings on session III. Data are reported as mean  $\pm$  s.e.m. (standard error of the mean). Differences are considered to be significant at  $p < 0.05$ .

All the statistical analyses are carried out using the Statview<sup>®</sup> 4.1 package.

**ARCHIVES STATEMENT**

Raw data, protocol and final report are kept in the archives room of ETAP for five years and VHS-videotapes for one year after the delivery of the study report.

**EXPERIMENTAL PERIOD**

April - May, 1998.



**PROTOCOL APPROVAL**

***EFFECT OF THE PRODUCT "ING 912" INTRAPERITONEALLY ADMINISTERED ON THE  
CONDITIONED PLACE PREFERENCE IN THE MALE WISTAR RAT***

**ETAP-Ethologie Appliquée**

**INGREDIA**

M. MESSAOUDI  
Scientific Director

Representative:  
Protocol approval by:

Date: March 16, 1998

Date:

Signature

Signature

***STUDY PROPOSAL N° INGREDIA 3/0298/PN-EPC - MARCH 16, 1998***

***FINAL REPORT - STUDY N° INGREDIA 3/0298/PN-EPC - OCTOBER 26, 1998***  
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