Appendix [illegible]

STUDY REPORT

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PROTOCOL CODE: INGREDIA-8/100/Pathoanatomy

SPONSOR

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STUDY IN CONFORMITY WITH THE GOOD ANIMAL
EXPERIMENT PRACTICES

May 2001

EXPERIMENT PERIOD: February - April 2001

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STUDY OF THE EFFECTS OF THE "ING 911" HYDROLYSATE, GIVEN ORALLY TO WISTAR MOTHER RATS THROUGHOUT PREGNANCY, ON INTERNAL AND EXTERNAL MALFORMATIONS OF THE YOUNGS FROM THEIR LITTERS

AUTHOR

I, undersigned, declare that the work described in this report was completed under my responsibility and that this report accurately corresponds to the results obtained.
Moreover, I declare that the present study was carried out in accordance with the Standard Work Protocols of the Pathoanatomy Laboratory of the Faculty of Medicine of Nancy, H. Poincaré University and in accordance with the Good Laboratory Practices, including original document filing.

Vandoeuvre-lès-Nancy, May 11th, 2001

Professor F. PLENAT

1 – INTRODUCTION

At the request of INGREDIA, the Pathoanatomy Laboratory of the Faculty of Medicine of Nancy, H. Poincaré University, carried out a macroscopic and microscopic study in order to evaluate the anatomical effects of "ING 911" hydrolysate, given to female rats from Wistar strain throughout pregnancy, on internal and external malformations in young males and females from their litters.

A three weeks old male and female were randomly selected in every litter whose mother was treated with a 150 mg/kg daily dose of ING 911 hydrolysate throughout pregnancy. A male and a female of the same age originating from litters whose mothers received only 150 mg/kg dose of powdered skim milk, were used as controls.

2 - MATERIALS AND METHODS

2.1 - Animals

Sixteen nulliparous females from the Wistar strain (Breeding centre Iffa-Credo, 69-St-Germain sur l'Arbresle, France), 12 weeks old, were crossed with 16 weeks old males of the same strain. The day after fecundation, the pregnant females were divided into two treatment groups (n = 8): a group was treated with a 150 mg/kg daily dose of ING 911 hydrolysate throughout pregnancy (D1 to D20) and the other received the same dose of powdered skim milk for the same time (control group). At birth, the females together with their litters were coded and installed in type-F polycarbonate cages (48 x 27 x 20 cm, U.A.R., 91 - Epinay-Sur-Orge, France). After recording of their size, the litters were reduced to 8 youngs (4 males and 4 females) in order to study comparable-sized groups of litter.
Crosses, pregnancy follow-up, female treatments and the tests of physical and neuromotor development of the youngs during three weeks following birth were carried out by the Centre de Recherches ETAP-Ethologie Appliquée, 13, rue du Bois de la Champelle - F - 54500 Vandoeuvre-lès-Nancy (Dr. A. NEJDI).

2.2 - Experimental procedure

At the age of three weeks, 8 young male and 8 young female rats were randomly selected in the litters of both groups and transferred to the Pathoanatomy Laboratory of the Faculty of Medicine of Nancy in order to detect any possible internal and external organic malformations.

Variables studied:
- external general malformations;
- internal visceral malformations;
- skeletal malformations;
- histological examination.

3 - RESULTS

Table 1

General malformations						
Controls **Batches 02-03/2001**	Male No.1	Male No.2	Male No.3	Male No. 4	Female No. 5	Female No.6
Head - normal	YES	YES	YES	YES	YES	YES
Eyes - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Ears - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Mouth - normal - cleft lip - cleft palate	YES YES NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT	YES NOT NOT
Foreleg - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Rear legs - normal - number - fingers	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES	YES 2 YES
Anus - normal	YES	YES	YES	YES	YES	YES
Ext. genit. organs - normal	YES	YES	YES	YES	YES	YES
Tail - normal - size	YES 9 cm	YES 8 cm	YES 6.5 cm	YES 6.5 cm	YES 8 cm	YES 6.5 cm
General skeleton - ossification . normal . ahead	YES YES	YES YES	YES YES	YES YES	YES YES	YES YES
Ribs - number - layout	12 YES	12 YES	12 YES	12 YES	12 YES	12 YES
Visceral malformations						
Heart - normal - cavities	YES YES	YES YES	YES YES	YES YES	YES YES	YES YES
Lungs - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Kidneys - normal - number	YES 2	YES 2	YES 2	YES 2	YES 2	YES 2
Digestive system Liver Stomach Intestines	YES YES YES	YES YES YES	YES YES YES	YES YES YES	YES YES YES	YES YES YES

Table 2

General malformations						
Treated Batches 02-03/2001	Male No.7	Male No.8	Male No.9	Male No.10	Female No.11	Female No.12
Head						
- normal	YES	YES	YES	YES	YES	YES
Eyes						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Ears						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Mouth						
- normal	YES	YES	YES	YES	YES	YES
- cleft lip	NOT	NOT	NOT	NOT	NOT	NOT
- cleft palate	NOT	NOT	NOT	NOT	NOT	NOT
Foreleg						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES
Rear legs						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES
Anus						
- normal	YES	YES	YES	YES	YES	YES
Ext. genit. organs						
- normal	YES	YES	YES	YES	YES	YES
Tail						
- normal	YES	YES	YES	YES	YES	YES
- size	7 cm	7.5 cm	7.5 cm	7 cm	7.5 cm	7 cm
General skeleton						
- ossification	YES	YES	YES	YES	YES	YES
. normal	YES	YES	YES	YES	YES	YES
. ahead						
Ribs						
- number	12	12	12	12	12	12
- layout	YES	YES	YES	YES	YES	YES
Visceral malformations						
Heart						
- normal	YES	YES	YES	YES	YES	YES
- cavities	YES	YES	YES	YES	YES	YES
Lungs						
- normal	YES	Left pulmonary lobe fissure beginning	YES	3 left lobes	YES	YES
- number	2	2	2	2	2	2
Kidneys						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Digestive system						
Liver	YES	YES	YES	YES	YES	YES
Stomach	YES	YES	YES	YES	YES	YES
Intestines	YES	YES	YES	YES	YES	YES

Table 3

General malformations							
Treated Batches 02-03/2001	Female No.13	Female No.14	Male No.21	Male No.22	Female No.23	Female No.24	Female No.25
Head							
- normal	YES	YES	YES	YES	YES	YES	YES
Eyes							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
Ears							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
Mouth							
- normal	YES	YES	YES	YES	YES	YES	YES
- cleft lip	NOT	NOT	NOT	NOT	NOT	NOT	NOT
- cleft palate	NOT	NOT	NOT	NOT	NOT	NOT	NOT
Foreleg							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES	YES
Rear legs							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES	YES
Anus							
- normal	YES	YES	YES	YES	YES	YES	YES
Ext. genit. organs							
- normal	YES	YES	YES	YES	YES	YES	YES
Tail							
- normal	YES	YES	YES	YES	YES	YES	YES
- size	7.5 cm	7.5 cm	8 cm	8 cm	9.3 cm	8.4 cm	8.5 cm
General skeleton							
- ossification	YES	YES	YES	YES	YES	YES	YES
. normal	YES	YES	YES	YES	YES	YES	YES
. ahead							
Ribs							
- number	12	12	12	12	12	12	12
- layout	YES	YES	YES	YES	YES	YES	YES
Visceral malformations							
Heart							
- normal	YES	YES	YES	YES	YES	YES	YES
- cavities	YES	YES	YES	YES	YES	YES	YES
Lungs							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
Kidneys							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
Digestive system							
Liver	YES	YES	YES	YES	YES	YES	YES
Stomach	YES	YES	YES	YES	YES	YES	YES
Intestines	YES	YES	YES	YES	YES	YES	YES

Table 4

General malformations						
Controls Batches 02-03/2001	Male No.15	Male No.16	Male No.17	Male No.18	Female No.19	Female No.20
Head						
- normal	YES	YES	YES	YES	YES	YES
Eyes						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Ears						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Mouth						
- normal	YES	YES	YES	YES	YES	YES
- cleft lip	NOT	NOT	NOT	NOT	NOT	NOT
- cleft palate	NOT	NOT	NOT	NOT	NOT	NOT
Foreleg						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES
Rear legs						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES
Anus						
- normal	YES	YES	YES	YES	YES	YES
Ext. genit. organs						
- normal	YES	YES	YES	YES	YES	YES
Tail						
- normal	YES	YES	YES	YES	YES	YES
- size	8 cm	8 cm	7.9 cm	8.1 cm	8 cm	8.2 cm
General skeleton						
- ossification	YES	YES	YES	YES	YES	YES
. normal	YES	YES	YES	YES	YES	YES
. ahead						
Ribs						
- number	12	12	12	12	12	12
- layout	YES	YES	YES	YES	YES	YES
Visceral malformations						
Heart						
- normal	YES	YES	YES	YES	YES	YES
- cavities	YES	YES	YES	YES	YES	YES
Lungs						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Kidneys						
- normal	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2
Digestive system						
Liver	YES	YES	YES	YES	YES	YES
Stomach	YES	YES	YES	YES	YES	YES
Intestines	YES	YES	YES	YES	YES	YES

Table 5

General malformations							
	Treated - Batches 04/2001			Controls - Batches 04/2001			
	Male No.1	Male No.2	Female No.3	Female No.1	Female No.2	Female No.3	Female No.4
Head							
- normal	YES	YES	YES	YES	YES	YES	YES
Eyes							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
Ears							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
Mouth							
- normal	YES	YES	YES	YES	YES	YES	YES
- cleft lip	NOT	NOT	YES	NOT	NOT	NOT	NOT
- cleft palate	NOT	NOT	NOT	NOT	NOT	NOT	NOT
Foreleg							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES	YES
Rear legs							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
- fingers	YES	YES	YES	YES	YES	YES	YES
Anus							
- normal	YES	YES	YES	YES	YES	YES	YES
Ext. genit. organs							
- normal	YES	YES	YES	YES	YES	YES	YES
Tail							
- normal	YES	YES	YES	YES	YES	YES	YES
- size	9.5 cm	8.5 cm	7 cm	7 cm	7 cm	8.2 cm	10 cm
General skeleton							
- ossification	YES	YES	YES	YES	YES	YES	YES
. normal	YES	YES	YES	YES	YES	YES	YES
. ahead							
Ribs							
- number	12	12	12	12	12	12	12
- layout	YES	YES	YES	YES	YES	YES	YES
Visceral malformations							
Heart							
- normal	YES	YES	YES	YES	YES	YES	YES
- cavities	YES	YES	YES	YES	YES	YES	YES
Lungs							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
Kidneys							
- normal	YES	YES	YES	YES	YES	YES	YES
- number	2	2	2	2	2	2	2
Digestive system							
Liver	YES	YES	YES	YES	YES	YES	YES
Stomach	YES	YES	YES	YES	YES	YES	YES
Intestines	YES	YES	YES	YES	YES	YES	YES

4 – CONCLUSION

No significant malformation was observed during macroscopic examination under operating microscope in young male and female rats of both treatment groups.

An extra pulmonary fissure observed in a male rat of the treated group cannot be regarded as a malformation, but corresponds in fact to a variation, within the normal limits.

Histological examinations relating to all the internal organs of control and treated rats of February – Mars 2001 series did not show tissue abnormalities.

The skeleton of two male rats (rats No. 8 and 10 of the group "Treated: February - Mars 2001 batches" was coloured with Alizarin and then diaphanised. No skeletal abnormality was observed.

Vandoeuvre-lès-Nancy, May 11th, 2001

Professor F. PLENAT