

Date :16/01/03	Nut (please do not fill out) <input type="checkbox"/>
Our reference : Desmodium	Pl (please do not fill out) <input type="checkbox"/>

## NOTIFICATION FILE PLANTS / NUTRIENTS

### 1. COMPANY :

Name : Biodynamics NV  
Address : J. Plateaustraat 4 8400 Oostende Belgium  
tel : 0032 (0)59 80.58.24 --- fax : 0032 (0)59 80.70.83  
Statute : Public Limited Company

### 2. NAME OF THE PRODUCT :

Trade name : Desmodium  
Our code : 2326  
Scientific name : -

### 3. NATURE OF THE PRODUCT :

- a) Presentation : plastic bottle
- b) Packaging : 200 ml
- c) Recommended daily allowance : 1 to 2 coffee spoon before meal

### 4. INGREDIENTS LIST :

- Active ingredients

Scientific name	Dutch name	French name	Part used	Galenic form	Amount
Desmodium adscendens			overground stems	liquid	75 g / 150 ml

- Non-active ingredients

Scientific name	Amount
Glycerin	13.6 g
Potassiumsorbate	2 g
Citric acid	0.024 g
Rozemary essential oil	0.066 ml

The part used and the galenic form only need to be mentioned for plants.

### 5. NUTRITIONAL ANALYSIS :

Energy in kcal	49 kcal /100 g
Energy in kJ	205 kJ /100 g
Carbohydrates	11 g /100 g
Proteins	1g /100 g
Lipids	0 g /100 g

**6. NUTRITIONAL VALUE :**

<b>Plants / Nutrients</b>	<b>Per recommended daily portion</b>	<b>Per 100 ml</b>
Desmodium adsendens	2.5 – 5 g (= 5 ml – 10 ml)	50 g

**7. ACTIVE SUBSTANCES OR MARKERS (only for plants) :**

<b>Active substances</b>	<b>Amount per daily portion</b>	<b>Amount per 100 g</b>
Saponin: dehydrosojsaponine Soyasaponine I and II Trytamine flavonoid	0.25 – 0.5 mg	5 g

**8. TOXICITY :**

No toxicity found in this recommended daily allowance.

**9. STABILITY :**

We guarantee the stability of the ingredients and active substances until the minimum expiry date.

**10. LABELLING OF THE PRODUCT :**

See enclosure

We commit ourselves to carrying out analyses frequently and on variable moments and to keeping the results at the General Food Inspection's disposal.
---

Ostend, 16/01/03,

Francis Maes  
Delegate Manager

S  
400433  
073266  
A

Do not use if seal around cap is broken or missing

**DIRECTIONS FOR USE:**

1 to 2 teaspoons before each meal, in a half glass of water, sugared or not

Refrigerate after opening.

Product number : 2326

Manufactured in E.C.

Packaged by BIODYNAMICS NV,

J. Plateaustr. 4- 8400 Oostende, Belgium

Distributed by MAES CENTER Dispensary,

19 E. Mission St., Suite A

Santa Barbara, CA 93101 USA

Lot: 2000828

Best before: 04/2003

# Desmodium

*Flavoured drinkable solution  
of the Desmodium adscendens*

DIETARY SUPPLEMENT

Biodynamics

**SHAKE WELL BEFORE USING**

200 ml of the solution correspond with 100 g of the plant

**Supplement Facts**

Serving size: 1 Teaspoon

Servings per container: 40

Amount Per Serving

Desmodium of the Desmodium: 5.0g

Calories: 40 KCal

Total carbohydrates: 10g/100g

Protein: 10g/100g

Salt: 10g/100g

Other ingredients: Glycerin, Potassium citrate

Lactic acid, Rosemary essence

Contains no yeast, wheat, salt, starch,

gluten or sugar

INFO: [www.maescenter.com](http://www.maescenter.com)

5.20 FL OZ / 200 ml Net

# CIT

DESMODIUM ADSCENDENS

MICRONUCLEUS TEST ON THE MOUSE

**CENTRE INTERNATIONAL DE TOXICOLOGIE  
(INTERNATIONAL TOXICOLOGY CENTRE)**

SEREY / BP 563 / 27005 ÉVREUX CEDEX FRANCE / TELEX 172046 F / FAX : +33 / 32 67 87 05

CONFIDENTIAL

DESMODIUM ADSCENDENS

MICRONUCLEUS TEST ON THE MOUSE

**ADDRESSEE:**

J. Ragot  
Centre de Recherches Phytothérapiques  
(Centre of Phytotherapeutic Research)  
23, Z.I. VIC  
Route d'Escalquens  
31320 Castanet-Tolosan

**DATE :**

December 15, 1989

**SUMMARY**

Title page	2
Summary	3
Attestation of the Study Director	4
Scientists participating in this study	5
Unity of Quality Assurance – Review	6

**SUMMARY**

7

**1. INTRODUCTION**

8

**2. MATERIALS AND METHODS**

9

**2.1. PRODUCTS**

9

## 2.1.1. Tested substance

9

## 2.1.2. Vehicle

9

## 2.1.3. Positive check sample

8

**2.2. ANIMALS**

10

## 2.2.1. Environment

10

## 2.2.2. Food and drink

10

**2.3. TREATMENT**

11

## 2.3.1. Method of administration

11

## 2.3.2. Posology

11

## 2.4. SAMPLING TIME AND SLIDES PREPARATION

11

## 2.5. LECTURE AND EVALUATION OF THE RESULTS

11

**3. RESULTS**

13

**4. CONCLUSION**

13

Tables 1 to 4

14-16

**5. BIBLIOGRAPHY**

17

**6. ARCHIVES**

17

**ANNEX**

18

**Analysis certificate**

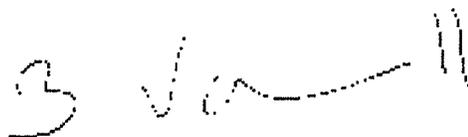
19-20

**ATTESTATION OF THE STUDY DIRECTOR**

This study was carried out in conformity with the protocol established with the Centre de Recherches Phytotherapiques (Centre of Phytotherapeutic Research) and following the rules of Good Laboratory Practice (Instruction No. 1065, May 31, 1983, Health Ministry, France).

I guarantee that this report is a true and sincere description of the applied procedures and of the results obtained in carrying out the study.

This study was carried out at the Centre International de Toxicologie (C.I.T.) in Miserey, 27005 Evreux, France.



-----  
B. Vanrell     Date: 15.12.89  
Doctor in Toxicology  
Responsible of the Mutagenesis Service  
Study Director

SCIENTISTS PARTICIPATING IN THIS STUDY

R. J. B. Vanrell

---

J. Bonafous    Date: 15.12.89  
National Diploma in Laboratory  
of Medical Analyses

B. Vanrell

---

B. Vanrell    Date: 15.12.89  
Doctor in Toxicology  
Responsible for of the Mutagenesis Service

R. Glomot / J.F. Le Bigot

---

R. Glomot / J.F. Le Bigot    Date : 15.12.89  
Scientific Management

UNITY OF QUALITY ASSURANCE – REVIEW

The protocol, the execution of the main study and the report were inspected by the Quality Assurance Unit of the International Toxicology Centre on the following dates:

<u>INSPECTION</u>	<u>INSPECTION DATE</u>	<u>INSPECTION REPORT DATE</u>
Protocol	03.08.89	03.08.89
Study	21.09.89	21.09.89
Report 1 <sup>st</sup> version	08.11.89	08.11.89
Final report	15.12.89	15.12.89

The inspections were carried out according to the C.I.T.-procedures and Good Laboratory Practice (Instruction No. 1065, 31 May 1983, Health Ministry, France).



---

H. Rault      Date: 15.12.89  
Responsible of the Quality Assurance Unit  
and of the Scientific Archives

### SUMMARY

The possible capacity of the product **DESMODIUM ADSCENDENS** to induce the micronuclei, was examined on the level of the cells of the bone marrow of the mouse.

The mice received one sole administration of the product in a dose of 5000 mg/kg orally. The count of micronucleus polychromatic erythrocytes was effectuated in the cells of the bone marrow, collected at three sacrifice times of 24, 48 and 72 hours after treatment. The control animals were treated with the vehicle (distilled water). The animals treated with 50 mg/kg cyclophosphamide, given orally, and sacrificed in the 24 hours period, formed the positive experimental animals.

The cyclophosphamide induces a statistically significant increase ( $p < 0,001$ ) in the number of micronuclei in the polychromatic erythrocytes, which clearly demonstrates the sensibility of the experiment under the study conditions. The statistically significant diminution ( $p < 0,05$ ) of the EP/EN proportion indicates a cytotoxic effect of the CPA with regard to the cells of the bone marrow.

At the three sacrifice times, the number of micronucleate polychromatic erythrocytes in the mice treated with the product **DESMODIUM ADSCENDENS**, does not differ in a statistically significant way from the number obtained with the vehicle-control animals.

Under these experimental conditions, the product **DESMODIUM ADSCENDENS** is not clastogenic in the micronucleus test on the mouse.

## 1. INTRODUCTION

The micronucleus test makes it possible to detect in vivo the chromosomic damages or these of the fusarial device, induced by the clastogenic agents.

This test is based on an increase in the number of micronuclei observed in the polychromatic erythrocytes of the bone marrow of the mice exposed to a clastogenic product compared with the control mice. The chromosomic fragments that have not migrated in a normal way during the mitosis, are not integrated in the cores of the daughter cells, but remain in the cytoplasm where they condense and form a parcel spheroid, called micronucleus. They can easily be detected in the polychromatic erythrocytes because the cells expulse their cores through erythropoiesis.

The agents that inhibit the proliferation or the maturation of the erythroblasts and these who destroy the nucleate cells, diminish the level of polychromatic erythrocytes (EP) in comparison with the normochromatic erythrocytes (EN), the polychromatophiles being distinguished from the normochromatophiles by their blueish colour. This way, the medullary cytotoxicity of a product can be determined by a decrease in the supply of EP/EN.

This test was carried out according to the technique described by Schmid (1) and modified by Salamone (2).

This study was effectuated because the micronucleus test is one of the tests proposed in Annex II of the Recommendation of the European Council No. 87/176/EEC of February 9, 1987 about the evaluation of the mutagenic power of pharmaceutical products, in the application of the Directive 75/318/EEC of May 20, 1975.

This protocol was established according to the OECD Guideline for the tests on chemical products, No. 474 "Genetic Toxicology: test of the micronucleus" of May 6, 1983.

This study was carried out according to the Good Laboratory Practice (Instruction No. 1065, May 31, 1983, Health Ministry, France).

## 2. MATERIALS AND METHODS

### 2.1. PRODUCTS

#### 2.1.1. Tested substance

The product **DESMODIUM ADSCENDENS** is a mixture, consisting of a powder of leaves, stems and aerial parts that form the non-blossoming anatomical parts of a plant of the papillonacea family. 10 g in a plastic phial and 2 g in a glass phial were received at the C.I.T. on July 21, 1989 under the reference "DESMODIUM ADSCENDENS, lot No. 079". 2 g in a glass phial were received at the C.I.T. on September 15, 1989 under the reference "DESMODIUM, July 89, lot No. 079". The product was conserved at environmental temperature. It was used in hydrodecoction obtained by boiling during 15 minutes 2 grams of crushed plant in 100 ml of distilled water; this solution was filtered on filter paper, then on millipore membrane 0,45 micron. This decoction was conserved during 24 hours at +4°C before being administered to the animals.

#### 2.1.2. Vehicle

Distilled water

#### 2.1.3. Positive control animal

Cyclophosphamid (CPA = Endoxan-Asta, lot No. 311, Laboratory Lucien (92700 Colombes, France) dissolved in distilled water at a concentration of 2,5 mg/ml.

## 2.2. ANIMALS

70 mice + 6 supplementary animals (38 males, 38 females), strain Swiss Cr1 : Cd-1 (1 CR) BR provided by Charles River France (76410 Saint Aubin les Elbeuf), were used. The animals, aged five weeks at their reception at the C.I.T., were observed during the 5 days of the acclimation period and after treatment.

Right before the treatment, they are individually identified by perforations or notches at the auricle. After randomisation, 7 groups composed of 10 animals (5 males, 5 females): 3 groups of vehicle control animals, 3 groups of treated animals, 1 group of positive control animals and 1 treated group composed of 6 supplementary animals (3 males, 3 females) were formed.

The last of these groups was sacrificed by asphyxia with CO<sub>2</sub> without removing any marrow, because no mortality intervened after the treatment.

At the day of the treatment, the animals weigh between 26, 6-31, 5 g for the males and between 22, 6-26,9 g for the females.

### 2.2.1. Stabling conditions

The study was carried out in an animal house with the following characteristics:

- temperature:  $21 \pm 2^{\circ}\text{C}$ ,
- hygrometry:  $50 \pm 20\%$ ,
- dial: 12h of light/ 12h of darkness
- air filtered by passage through absolute filter and non-recycled

The animals are harboured as follows: five of the same sex and of the same group (3 for the supplementary animals) in polycarbonate cages with a cover of stainless steel, containing autoclaved wood sawdust.

### 2.2.2. Food and drink

The animals have free access to rat and mouse food U.A.R. (ref. A04 C, U.A.R., 91360 Villemoisson sur Orge, France) and to drinking water filtered on millipore membrane 0,22 micron. Bacteriological and chemical analyses of the drinking water and of the main contaminants are carried out on a regular basis by the Municipal Laboratory, 76000 Rouen, France. The Study Director was not provided with any information indicating the presence in the food or in the drinking water of substances likely to interfere with the results of the study.

### **2.3. TREATMENT**

#### **2.3.1. Method of administration**

The vehicle, the **DESMODIUM ADSCENDENS** and the CPA were administered orally in a dose of 20 ml/kg.

#### **2.3.2. Posology**

As a result of the preliminary study, the **DESMODIUM ADSCENDENS** was administered in doses of 1000; 2500 and 5000 mg/kg.

As a result of the cytogenetic study, the **DESMODIUM ADSCENDENS** was administered one time in a dose of 5000 mg/kg and the cyclophosphamid in a dose of 50 mg/kg.

### **2.4. SAMPLING TIME AND SLIDES PREPARATION**

The 3 sampling times of 24, 45 and 72 hours were selected because they are recommended in the OECD guidelines.

The mice were sacrificed by asphyxia with CO<sub>2</sub>. The femurs were afterwards removed and the bone marrow extracted and mixed with the foetal calf serum. The cells were then centrifuged, the supernate was eliminated and the cells of the rest were put back into suspension by agitation. One drop of this cellular suspension was placed and displayed on a precoded slide. The slides/animal were prepared, but one was saved for the lecture.

## **2.5. LECTURE OF THE SLIDES AND EVALUATION OF THE RESULTS**

All the coded slides were read under the microscope at a large magnification (x 1000).

For each mouse, the micronuclei were counted in 1000 polychromatic erythrocytes. For every sacrifice time, the average number of micronucleate polychromatic erythrocytes (MPE/EP) in each treated group was compared with the one of the corresponding vehicle group. The statistic analysis was carried out with the Kastenbaum and Bowman test (4), for which  $p < 0,05$  represents the weakest significant probability.

The link between the polychromatic (EP) and the normochromatic erythrocytes (EN) is determined at a total of 1000 erythrocytes. This link (EP/EN) enables us to evaluate the medullary cytotoxicity induced by products, by using the statistic "t" test of Student.

Under our experimental conditions, a product is considered clastogenic:

- if it induces a statistically significant increase in the number of micronuclei (EPM/PE) and at least one of the sacrifice times compared with the vehicle group,
- and if this increase reaches the doubling of the EPM/PE-link of our historical check values ( $1,6 \pm 0,5$  0/00), i.e. if the link is more than 3,2 0/00.

In this case, an additional study should be carried out in order to indicate a possible relation dose-effect. This complementary study was effectuated with 3 product doses and at the sacrifice time for which the increase in EPM/PE-link was registered.

### 3. RESULTS

#### 3.1. PRELIMINARY STUDY

In order to determine the dose of the product **DESMODIUM ADSCENDENS** to administrate as a result of the cytogenetic study, the dose of 5000 mg/kg was administered orally to a group of 3 males and 3 females.

Not any symptomatology was observed after treatment.

The dose of 5000 mg/kg was then retained because it is the maximum dose recommended by the OECD.

#### 3.2. CYTOGENETIC STUDY

The summary of the examinations on bone marrow is shown in table 1 and their individual values in tables 2 to 4.

In the vehicle groups, the average values of the micronucleate polychromatic erythrocytes for 1000 polychromatic erythrocytes (EPM/PE) are 1,6; 0,8 and 1,3 respectively at the times 24, 48 and 72 hours after treatment.

In all the groups treated with the product **DESMODIUM ADSCENDENS**, the average values of EMP/PE-link are equivalent to those of their corresponding vehicle groups. Moreover, the PE/EN-links of the treated animals are of the same order of magnitude as those of the vehicle groups.

The treatment with cyclophosphamid induces a statistically significant increase ( $p < 0,001$ ) in the number of EPM/PE compared with the vehicle control group of 24 hours ( $29,7 \pm 11,4$  o/oo vs.  $1,6 \pm 0,8$ ), which indicates the sensibility of the system under our experimental conditions. Moreover, the PE/EN-condition decreases in a statistically significant way ( $p < 0,05$ ), which demonstrates the medullary cytotoxicity of the CPA.

### 4. CONCLUSION

Under these experimental conditions, the product **DESMODIUM ADSCENDENS** is not clastogenic in the micronucleus test on the mouse.

TABLE 1

**TEST OF THE MICRONUCLEUS  
ACTIVITY OF THE DESMODIUM ADSCENDENS**

<b>Sacrifice time (hours)</b>	<b>treatment group</b>	<b>dose (mg/kg)</b>	<b>MPE o/oo PE average (standard deviation)</b>	<b>PE/NE-link average (standard deviation)</b>
24	VEHICLE	-	1.6 (0.8)	0.8 (0.2)
	DESMODIUM	5000	1.5 (1.8)	0.9 (0.2)
	CPA	50	29.7 (11.4) ***	0.6 (0.2) *
48	VEHICLE	-	0.8 (1.0)	0.9 (0.2)
	DESMODIUM	5000	1.5 (1.3)	0.9 (0.3)
72	VEHICLE	-	1.3 (1.6)	1.1 (0.3)
	DESMODIUM	5000	1.7 (1.5)	0.9 (0.3)

10 animals (5 males, 5 females) per treatment group

CPA: cyclophosphamid

MPE: micronucleate polychromatic erythrocytes

PE: polychromatic erythrocytes

NE: normochromatic erythrocytes

**Statistics tests used:**

Kastenbaum and Bowman: MPE o/oo EP

"t" test of Student: link EP/NE

statistically significant value compared with the vehicle check:

\* p < 0,05

\*\*\* p < 0,001

TABLE 2

## INDIVIDUAL VALUES 24 HOURS AFTER TREATMENT

VEHICLE						DESMODIUM ADSCENDENS					
Sex	animals	MPE/PE	PE	NE	P	sex	animals	MPE/PE	PE	NE	P
Male	01	1	433	567	0,8	Male	11	2	429	571	0,8
	02	1	372	628	0,6		12	1	550	450	1,2
	03	1	480	520	0,9		13	2	456	544	0,8
	04	1	508	492	1,0		14	1	377	623	0,6
	05	1	373	627	0,6		15	0	336	664	0,5
Female	06	3	537	463	1,2	Female	16	4	552	448	1,2
	07	3	432	568	0,8		17	0	473	527	0,9
	08	2	406	594	0,7		18	0	442	558	0,8
	09	2	373	627	0,6		19	5	458	542	0,8
	10	1	319	681	0,5		20	0	524	476	1,1
average		1,6			0,8	average		1,5			0,9
standard deviation		0,8			0,2	standard deviation		1,8			0,2

CYCLOPHOSPHAMID					
Sex	animals	MPE/PE	PE	NE	P
Male	21	24	358	642	0,6
	22	12	272	728	0,4
	23	23	500	500	1,0
	24	41	293	707	0,4
	25	51	300	700	0,4
Female	26	32	397	603	0,7
	27	23	464	536	0,9
	28	21	472	528	0,9
	29	37	352	648	0,5
	30	33	352	648	0,5
average		29,7			0,6
standard deviation		11,4			0,2

MPE/PE : micronucleate polychromatic erythrocytes / 1000 polychromatic erythrocytes

PE: polychromatic erythrocytes

NE: normochromatic erythrocytes

P: proportion PE/NE

TABLE 3

## INDIVIDUAL VALUES 48 HOURS AFTER TREATMENT

VEHICLE						DESMODIUM ADSCENDENS					
Sex	animals	MPE/PE	PE	NE	P	sex	animals	MPE/PE	PE	NE	P
Male	31	1	392	608	0,6	Male	41	4	323	677	0,5
	32	0	446	554	0,8		42	1	385	615	0,6
	33	0	564	436	1,3		43	0	556	444	1,3
	34	2	326	674	0,5		44	1	432	568	0,8
	35	1	524	476	1,1		45	3	469	531	0,9
Female	36	0	463	537	0,9	Female	46	0	494	506	1,0
	37	1	456	544	0,8		47	1	515	485	1,1
	38	0	450	550	0,8		48	2	508	492	1,0
	39	0	477	523	0,9		49	1	521	479	1,1
	40	3	427	573	0,7		50	2	549	451	1,2
average		0,8			0,9	average		1,5			0,9
standard deviation		1,0			0,2	standard deviation		1,3			0,3

TABLE 4

## INDIVIDUAL VALUES 72 HOURS AFTER TREATMENT

VEHICLE						DESMODIUM ADSCENDENS					
Sex	animals	MPE/PE	PE	NE	P	sex	animals	MPE/PE	PE	NE	P
Male	51	0	530	470	1,1	Male	61	4	447	553	0,8
	52	0	443	557	0,8		62	1	322	678	0,5
	53	5	528	472	1,1		63	3	326	674	0,5
	54	0	433	567	0,8		64	0	524	476	1,1
	55	1	621	379	1,6		65	2	527	473	1,1
Female	56	0	529	471	1,1	Female	66	1	501	499	1,0
	57	2	492	508	1,0		67	1	420	580	0,7
	58	1	597	403	1,5		68	0	576	424	1,4
	59	1	448	552	0,8		69	4	500	500	1,0
	60	3	553	467	1,1		70	1	438	562	0,8
average		1,3			1,1	average		1,7			0,9
standard deviation		1,6			0,3	standard deviation		1,5			0,3

MPE/PE : micronucleate polychromatic erythrocytes / 1000 polychromatic erythrocytes

PE: polychromatic erythrocytes

NE: normochromatic erythrocytes

P: proportion PE/NE

## 5. BIBLIOGRAPHY

1. Schmid, W.  
The micronucleus test.  
Mutation Study, 31, 1975, 9-15
2. Salamone M., Heddle J., Stuart E. and Katz M.  
Toward an improved micronucleus test. Studies on three model agents, mitomycin C, CPA and dimethylbenzanthracene.  
Mutation Research, 74, 1980, 347-356
3. Heddle J. and Salamone M.  
The micronucleus test. I. in vivo.  
In: short term test for chemical carcinogens.  
Springer Verlag New York (Stich and San eds.), 1981, 243-249.
4. Kastenbaum M.A. and Bowman K.O.  
Tables for determining the statistical significance of mutation frequencies  
Mutation Research, 9, 1970, 527-549.

## 6. ARCHIVES

The following documents:

- the study protocol and their possible amendments,
- the study register,
- the correspondence,
- the study report (final) and their possible amendments,
- the slides,

are kept in the archives of the International Toxicology Centre in Miserey, 27005 Evreux, France, for a period of 5 years after the end of the study in vivo. After this term, the study archives are, to the choice of the mandatory laboratory, either transferred to their quarters, or destroyed.

ANNEX

ANALYSES	RESULTS	NORMS
Visual research of other substances and moulds	absence	absence
<u>Identification</u>		
- Macroscopic aspect	conform	
- Microscopic aspect after colouring the transversal sections of leaves and stems	conform	identical to the samples already identified
<u>Doses</u>		
- Humidity percentage	9%	IO $\pm$ 2%
- Ash percentage	7,1%	7 $\pm$ 1%
- Indolic alkaloids proportion expressed in comparison with the tryptamine	4 mg/kg	< 25 $\mu$ g/l decoction 4 $\pm$ 0,4 mg/kg plants
- Organophosphors	not detected	absence
- Organochlores (Alpha Lindane) (Lindane)	20 mg/t 15 mg/t	20 mg/ton 20 mg/ton

# CIT

DESMODIUM ADSCENDENS

EXPERIMENT OF REVERSE MUTATION  
THROUGH THE AMES TEST

**CENTRE INTERNATIONAL DE TOXICOLOGIE**  
**(INTERNATIONAL TOXICOLOGY CENTRE)**

SEREY / BP 563 / 27005 ÉVREUX CEDEX FRANCE / TELEX 172046 F / FAX : +33 / 32 67 87 05

CONFIDENTIAL

DESMODIUM ADSCENDENS

EXPERIMENT OF REVERSE MUTATION  
THROUGH THE AMES TEST

**ADDRESSEE:**

J. Ragot  
Centre de Recherches Phytothérapiques  
(Centre of Phytotherapeutic Research)  
23, Z.I. VIC  
Route d'Escalquens  
31320 Castanet-Tolosan

**DATE :**

January 8, 1990

**SUMMARY**

Title page	2
Summary	3
Attestation of the Study Director	4
Scientists participating in this study	5
Unity of Quality Assurance – Review	6

**SUMMARY**

7

**1. INTRODUCTION**

8

**2. MATERIAL AND METHODS**

9

2.1. Bacterial strains	9
2.2. Metabolic activation system in vitro : S9 mix	10
2.3. Products	10
2.3.1. Tested substance	10
2.3.2. Positive experimental group	11
2.4. Experimental procedure	11
2.4.1. Toxicity test	12
2.4.2. Mutagenesis test	12
2.5. Evaluation of the results	12

**3. RESULTS**

13

3.1. Toxicity	13
3.2. Mutagenic activity	13

**4. CONCLUSION**

13

Tables 1 to 5	14-18
---------------	-------

**5. BIBLIOGRAPHY**

19

**6. ARCHIVES**

19

**ANNEX**

20

**Analysis report**

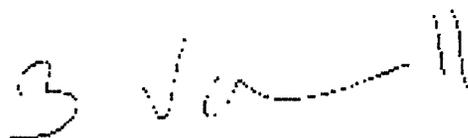
21-22

**ATTESTATION OF THE STUDY DIRECTOR**

This study was carried out in conformity with the protocol established with the 'Centre de Recherches Phytotherapic' (Centre of Phytotherapeutic Research) and following the rules of Good Laboratory Practice (Instruction No. 1065, Health Ministry, France, May 31, 1983).

I guarantee that this report is a true and sincere description of the applied procedures and of the results obtained in the execution of the study.

This study was carried out at the Centre International de Toxicologie (C.I.T.) in Miserey, 27005 Evreux, France.



---

B. Vanrell      Date: 08.01.89  
Doctor in Toxicology  
Responsible of the Mutagenesis Service  
Study Director

SCIENTISTS PARTICIPATING IN THIS STUDY

~~1/11/90~~

---

S. Thenaisie Date: 08.01.90  
Diploma of higher education of technician  
in Biological Analyses

R. B. Vanrell

---

M. N. Gascoin Date: 08.01.90  
Diploma of Advanced Studies  
in Cellular and Molecular Biology

B. Vanrell

---

B. Vanrell Date : 08.01.90  
Doctor in Toxicology  
Responsible of the Mutagenesis service



---

R. Glomot / J.F. Le Bigot Date : 08.01.90  
Scientific Management

UNITY OF QUALITY ASSURANCE – REVIEW

The protocol, the execution of the main study and the report were inspected by the Quality Assurance Unit of the Centre International de Toxicologie on the following dates:

<u>INSPECTION</u>	<u>INSPECTION DATE</u>	<u>INSPECTION REPORT DATE</u>
Protocol	03.08.89	03.08.89
Study	29.08.89	31.08.89
Report 1 <sup>st</sup> version	12.12.89	14.12.89
Final report	08.01.90	08.01.90

The inspections were carried out according to the C.I.T.-procedures and the Good Laboratory Practice (Instruction No. 1065, Health Ministry, France, May 31, 1983).



---

H. Rault      Date: 08.01.90  
Responsible of the Quality Assurance Unit  
and of the Scientific Archives

**SUMMARY**

The mutagenic activity in vitro of the product **DESMODIUM ADSCENDENS** was determined in the Ames test on the five strains of Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100. This test enables us to detect the mutations by substitution of the base pairs or the mutations by shifting the genetic code reading frame.

After a preliminary experiment, defining the doses to be used for the mutagenesis test, the product was tested through two independent experiments. Each experiment was effectuated both with and without a metabolisation system, the S9 mix, prepared from a microsomial hepatic fraction of rats pretreated with Aroclor 1254. The technique used is the incorporation in the Petri dish. The concentrations are 100 and 5000 µg/dish.

The product **DESMODIUM ADSCENDENS** does not induce any statistically significant augmentation in the number of reverse mutants, both with and without the system of metabolic activation S9 mix on the 5 used strains.

The number of reverse mutants induced by the reference mutagenic products is clearly superior to the number of spontaneous and/or solvent reverse mutants, which indicates the sensibility of the test as well as the efficiency of the metabolisation system.

Under these experimental conditions, the product **DESMODIUM ADSCENDENS** is not mutagenic in the Ames test.

## 1. INTRODUCTION

The back mutation test in vitro on the bacterium Salmonella typhimurium, finalized by Ames and Coll. (1,2), makes it possible to detect the acting mutagenic products, either by substitution of the base pairs or by shifting the reading frame of the genetic code.

The mutagenic products induce back mutations of the auxotrophic bacteria for the histidine, who then become capable of developing on an environment limiting in histidine, while the non-mutated bacteria do not develop on it.

The study was effectuated through the direct incorporation method in the Petri dish (4), both with and without a metabolist activation system. It also makes it possible to determine whether the tested product and/or its possible metabolites present a mutagenic effect.

This test was carried out because the Ames test is one of the tests proposed in Annex II of the Recommendation 87/176/EEC of the European Council concerning the mutagenesis experiments of pharmaceutical products, for the application of Directive 75/318/EEC of May 20, 1975.

This protocol was established according to the OECD Guideline No. 471 "Genetic Toxicology: Back Mutation Experiment on Salmonella tiphymurium" of May 26, 1983.

## 2. MATERIAL AND METHODS

### 2.1. BACTERIAL STRAINS

The 5 used strains: TA 1535, TA 1537, TA 1538, TA 98 and TA 100, come from the Laboratory of B.N. Ames (University of California, Berkeley, U.S.A.). They are conserved at  $-80^{\circ}\text{C}$  in sterile tubes containing 1 ml of enrichment broth and 0,09 ml of dimethylsulfoxyde. On the eve of the experiments, a well isolated colony coming from the master dishes was sterilely detracted and sown in around 5 ml of enrichment broth; the whole was afterwards put at  $37^{\circ}\text{C}$  under agitation during approximately 14 hours.

Created from the primitive genotype of Salmonella typhimurium LT2, the strains contain a mutation at the level of the histidine operon, which makes them autotrophic to this amino acid. What is more, in order to increase their sensibility to mutagenic agents, additional mutations were created:

- the rfa mutation alters the lypopolysaccharidic barrier of the bacteriological cell wall and this way shows a larger membrane permeability.
- a deletion in the gene uvr B eliminates the repair system by excision; this way the damages caused by a mutagenic agent are not repaired anymore by the bacterium and can be expressed at the level of the phenotype,
- the addition in the strains TA 98 and TA 100 of a resistance factor R to the ampicillin from the plasmid pKM 101, increases the detection sensibility of certain mutagens.

#### Genotype of the bacterial strains

<b>Strains</b>	<b>histidine mutation</b>	<b>additional mutations</b>
TA 1535	his G 46	rfa    uvr B
TA 100	his G 46	rfa    uvr B    Factor R
TA 1537	his C 3076	rfa    uvr B
TA 1538	his D 3052	rfa    uvr B
Ta 98	his D 3052	rfa    uvr B    Factor R

The strains TA 1535 and TA 100 detect the mutagenic substances by substituting the base pairs; the strains TA 1537, TA 1538 and TA 98 detect the "frameshift" type mutagens by shifting the reading frame of the genetic code.

The specific characters on each strain are verified periodically, every 2 months  $\pm$  (2 weeks).

## **2.2. METABOLIC ACTIVATION SYSTEM IN VITRO: S9 MIX**

The S9 mix consists of induced enzymatic systems, contained in the microsomal fraction of the rat liver and of cofactors necessary for their functioning as far as this was described by Ames and Coll. (4).

In order to induce the enzymatic systems, Sprague-Dawley rats, aged 6 weeks and weighing approximately 200 grams (Charles River France, 76410 Saint Aubin les Elbeuf) are treated with Aroclor 1254 (500 mg/kg), administered through the peritoneum, five days before the sacrifice. The liver of the rats is then sterilely removed, washed in a sterile and cold solution of KCL 0,15M (+ 4°C) and weighed. Per gram of liver, three ml of KCL 0,15M are added, then the liver was crushed in a homogeniser; the homogenisate is centrifuged at 9000 g during 10 minutes at + 4°C. The supernate obtained forms the S9 fraction and is conserved in sterile tubes at - 80°C.

The S9 mix is prepared extemporaneously at + 4°C and conserved at this temperature during the experimentation. It contains per ml:

- 5 µmoles Glucose-6-Phosphate,
- 4 µmoles NADP,
- 33 µmoles KCl,
- 8 µmoles MgCl<sub>2</sub>,
- 100 µmoles sodium phosphate pH 7,4,
- 20 µl of S9 (lot No 28 ; lot No 29 for the experiment of October 25, 1989).

The activity of the S9 mixes was checked in advance and was found to be satisfying at 2 % of S9 in the S9 mix. The protein content of the S9 fraction is 32 g/l (lot No 28) or 27 g/l (lot No 29).

## **2.3. PRODUCTS**

### **2.3.1. Tested substance**

The product **DESMODIUM ADSCENDENS** looks like a powder of green leaves. It is a mixture of powder of green leaves, stems and non-blossoming aerial parts of a plant of the papillonacea family. 2 g in a glass phial and 10 g in a plastic phial were received at the C.I.T. on July 21, 1989 under the reference "DESMODIUM ADSCENDENS Lot 079". This product was conserved at environmental temperature according to the information provided by the Demand Laboratory. It was used in hydrodecoction obtained by boiling for 15 minutes 1 g of the crushed plant in 100 ml of distilled water (concentration: 10 mg/ml), then it was filtered on paper and on millipore membrane 0,45 micron. This decoction was used extemporaneously or after conservation at + 4°C for a maximum of 24 hours.

### 2.3.2. Positive experimental animals

These products were dissolved in dimethylsulfoxide (DMSO, lot No. 025250, Merck).

<b>Products</b>	<b>Concentrations (µg/dish)</b>	<b>Strains</b>
<u>Without S9 mix:</u>		
Sodium azide (NaN <sub>3</sub> ) (Sigma, lot No 53F-0702)	1	TA 1535 TA 100
9-aminoacridine (9AA) (Janssen, lot No 000601)	50	TA 1537
2-nitrofluorene (2NF) (Janssen, lot No 104 224 37)	0,5	TA 1538 TA 98
<u>With S9 mix:</u>		
2-anthramine (2AM) (Sigma, lot No 33F-0816)	2 1	TA 1535-TA 1537 TA 1538-TA 98-TA 100

## 2.4. EXPERIMENTAL PROCEDURE

The method applied is the one described by Maron and Ames (4), i.e. the incorporation in the Petri dish.

The solution of the product, of which the volume varies from 100 to 500 µg/dish, 0,5 ml S9 mix for the experiments with the metabolism system and 0,1 ml of the strain was added to 2 ml soft agar, containing traces of histidine and biotine and kept in supercooling at 45 °C. After rapid homogenisation, the mix is displayed in the Petri dish containing a minimum nutrient medium.

After 48 to 72 hours of incubation at 37°C, the counting of the colonies is effectuated with an automatic counter (Artek counter, model 880, OSI, 75015 Paris, France).

#### 2.4.1. Toxicity test

In order to determine the maximum concentration of the product that does not affect the growth of the bacteria, a range of five concentrations of the product is tested on two strains of TA 98 and TA 100 : 10, 100, 1000, 2500 and 5000 µg/dish. The toxic activity is evaluated by a clearing of the bacterial carpet due to an inhibition of the bacterial growth, and possibly by a diminution of the number of colonies.

The sterility of the product is verified with this experiment.

#### 2.4.2. Mutagenicity tests

As a result of two independent experiments, 5 concentrations of the product (3 dishes/concentration) are tested on the 5 strains with or without metabolic activation: 100 at 5000 µg/dish. As a result of each experiment, the spontaneous reversion experimental animals of the strains alone (negative experimental animals) and with the solvent (solvent experimental animals) are included. The sensibility of the strain and the metabolising power of the S9 mix are checked with mutagenic reference products (positive experimental animals). The sterility of the S9 mix is verified in each experiment, both at the start and at the end of the experimentation.

### **2.5. EVALUATION OF THE RESULTS**

The product is considered to be mutagenic if, for one of the strains,:

- it induces a doubling of the number of reverse mutants in comparison with the number of spontaneous and/or solvent mutants,
- and
- a statistically significant and reproducible relation dose - effect (linear regression, for which  $p < 0,05$  is used as the weakest possibility) is demonstrated.

### 3. RESULTS

#### 3.1. TOXICITY

The results concerning the toxic activity of the product **DESMODIUM ADSCENDENS** are presented in table 1.

There was not observed any toxic effect of the product regarding the strains TA 98 and TA 100.

#### 3.2. MUTAGENIC ACTIVITY

The results of the experiments are presented in the tables 2 to 5.

The concentrations removed for the experiments are 100 to 5000 µg/dish with or without S9 mix.

With regard to the five used strains, the number of reverse mutants with the product **DESMODIUM ADSCENDENS** is equivalent to the number of spontaneous and/or solvent experimental animals.

The number of spontaneous and solvent reverse mutants is equivalent to the number habitually obtained at the Laboratory. The number of reverse mutants induced by the mutagenic reference products is clearly superior to the number of spontaneous and/or solvent reverse mutants, which shows the sensibility of the test as a result of the experiments and the actual activation of the 2-Anthramine by the S9 mix.

### 4. CONCLUSION

Under these experimental conditions, the product **DESMODIUM ADSCENDENS** is not mutagenic in the Ames test.

TABLE 1  
**BACTERIOSTATIC ACTIVITY  
 OF THE PRODUCT: DESMODIUM ADSCENDENS**

Strains	concentrations (µg/dish)	toxicity	WITHOUT S-9 MIX		WITH S-9 MIX	
			reverse mutants per dish	toxicity	reverse mutants per dish	
TA 98	0	-	14	-	32	
	10	-	13	-	33	
	100	-	24	-	25	
	1000	-	20	-	31	
	2500	-	21	-	21	
	5000	-	20	-	19	
TA 100	0	-	93	-	95	
	10	-	85	-	85	
	100	-	82	-	86	
	1000	-	95	-	113	
	2500	-	78	-	88	
	5000	-	83	-	60	

toxicity

- : not any

+ : light

++ : middle

+++ : important to complete

0: solvent experimental animal

TABLE 2

**MUTAGENIC ACTIVITY OF THE PRODUCT:  
DESMODIUM ADSCENDENS  
WITHOUT METABOLIC ACTIVATION**

strains	concentrations (µg/dish)	reverse mutants per dish				middle	standard deviation	proportion
TA 1535	RS	14	15	10	13	3	-	
	0	9	10	9	9	1	-	
	100	10	5	6	7	3	0,8	
	500	6	7	8	7	1	0,8	
	1000	12	11	6	10	3	1,0	
	2500	8	7	5	7	2	0,7	
	5000	14	6	7	9	4	1,0	
	NaN3 (1)	362	371	354	362	9	38,8	
TA 1537	RS	18	13	10	14	4	-	
	0	18	11	17	15	4	-	
	100	16	13	17	15	2	1,0	
	500	20	17	20	19	2	1,2	
	1000	11	11	15	12	2	0,8	
	2500	20	12	19	17	4	1,1	
	5000	19	20	15	18	3	1,2	
	9AA (50)	178	152	343	224	104	14,6	
TA 1538	RS	29	21	19	23	5	-	
	0	22	17	11	17	6	-	
	100	17	14	15	15	2	0,9	
	500	20	15	18	18	3	1,1	
	1000	29	22	16	22	7	1,3	
	2500	15	19	26	20	6	1,2	
	5000	21	20	22	21	1	1,3	
	2NF (0'5)	201	188	185	191	9	11,5	
TA 98 *	RS	19	23	18	20	3	-	
	0	25	25	17	22	5	-	
	100	20	23	25	23	3	1,0	
	500	23	24	14	20	6	0,9	
	1000	28	25	22	25	3	1,1	
	2500	33	29	28	30	3	1,3	
	5000	17	19	26	21	5	0,9	
	2NF(0,5)	125	114	154	131	21	5,9	
TA 100	RS	101	98	76	92	14	-	
	0	93	80	72	82	11	-	
	100	89	72	80	80	9	1,0	
	500	72	89	71	77	10	0,9	
	1000	76	79	51	69	15	0,8	
	2500	80	84	86	83	3	1,0	
	5000	72	70	73	72	2	0,9	
	NaN3(1)	440	356	381	392	43	4,8	

RS : spontaneous reverse mutants                      0 : solvent experimental animal

Proportion:  $\frac{\text{number of reverse mutants with product}}{\text{number of reverse mutants with solvent}}$

TABLE 3

**MUTAGENIC ACTIVITY OF THE PRODUCT:  
DESMODIUM ADSCENDENS  
WITH METABOLIC ACTIVATION**

strains	concentrations (µg/dish)	reverse mutants per dish			middle	standard deviation	proportion
TA 1535	0	9	9	8	9	1	-
	100	7	11	8	9	2	1,0
	500	8	16	3	9	7	1,0
	1000	12	15	6	11	5	1,3
	2500	11	9	6	9	3	1,0
	5000	8	4	4	5	2	0,6
	2AM(2)	195	212	242	216	24	25,0
TA 1537	0	19	13	23	18	5	-
	100	16	12	12	13	2	0,7
	500	16	21	23	20	4	1,1
	1000	13	19	19	17	3	0,9
	2500	14	18	18	17	2	0,9
	5000	14	18	16	16	2	0,9
	2AM(2)	286	297	233	272	34	14,8
TA 1538	0	29	22	15	22	7	-
	100	23	17	15	18	4	0,8
	500	21	18	19	19	2	0,9
	1000	12	13	22	16	6	0,7
	2500	22	19	23	21	2	1,0
	5000	21	26	28	25	4	1,1
	2AM(1)	1113	924	849	962	136	43,7
TA 98*	0	17	25	22	21	4	-
	100	31	35	19	28	8	1,3
	500	32	18	26	25	7	1,2
	1000	26	26	34	29	5	1,3
	2500	21	22	24	22	2	1,0
	5000	22	22	29	24	4	1,1
	2AM(1)	1427	1487	1471	1462	31	68,5
TA 100	0	101	64	91	85	19	-
	100	61	65	68	65	4	0,8
	500	65	70	73	69	4	0,8
	1000	78	99	70	82	15	1,0
	2500	80	79	79	79	1	0,9
	5000	93	71	66	77	14	0,9
	2AM(1)	2151	1992	1746	1963	204	23,0

0 : solvent experimental animal

proportion:  $\frac{\text{number of reverse mutants with the product}}{\text{number of reverse mutants with the solvent}}$

Table 4

**MUTAGENIC ACTIVITY OF THE PRODUCT:  
DESMODIUM ADSCENDENS  
WITHOUT METABOLIC ACTIVATION**

Strains	concentrations (µg/dish)	reverse mutants per dish			middle	standard deviation	proportion
TA 1535	RS	5	10	4	6	3	-
	0	16	11	9	12	4	-
	100	7	5	10	7	3	0,6
	500	9	11	10	10	1	0,8
	1000	17	16	16	16	1	1,4
	2500	15	7	5	9	5	0,8
	5000	22	13	11	15	6	1,3
	NaN3 (1)	262	235	237	245	15	20,4
TA 1537	RS	2	2	2	2	0	-
	0	8	4	2	5	3	-
	100	5	8	6	6	2	1,4
	500	7	3	5	5	2	1,1
	1000	3	9	3	5	3	1,1
	2500	6	5	5	5	1	1,1
	5000	3	4	6	4	2	0,9
	9AA (50)	129	103	118	117	13	25,0
TA 1538	RS	31	26	27	28	3	-
	0	31	28	26	28	3	-
	100	29	33	22	28	6	1,0
	500	24	26	27	26	2	0,9
	1000	43	26	20	30	12	1,0
	2500	40	33	26	33	7	1,2
	5000	22	28	22	24	3	0,8
	2NF(0,5)	170	200	219	196	25	6,9
TA 98*	RS	38	32	37	36	3	-
	0	52	38	37	42	8	-
	100	30	44	44	39	8	0,9
	500	37	41	66	48	16	1,1
	1000	50	49	41	47	5	1,1
	2500	46	31	37	38	8	0,9
	5000	51	47	44	47	4	1,1
	2NF(0,5)	170	217	175	187	26	4,4
TA 100	RS	66	80	86	77	10	-
	0	72	68	78	73	5	-
	100	54	60	59	58	3	0,8
	500	61	83	66	70	12	1,0
	1000	58	75	57	63	10	0,9
	2500	68	49	68	62	11	0,8
	5000	61	62	64	62	2	0,9
	NaN3	356	350	341	349	8	4,8

RS: spontaneous reverse mutants

0: solvent experimental animal

Proportion:  $\frac{\text{number of reverse mutants with product}}{\text{number of reverse mutants with solvent}}$

Table 5

**MUTAGENIC ACTIVITY OF THE PRODUCT:  
DESMODIUM ADSCENDENS  
WITH METABOLIC ACTIVATION**

Strains	concentrations (µg/dish)	reverse mutants per dish			middle	standard deviation	proportion
TA 1535	0	5	11	14	10	5	-
	100	2	5	11	6	5	0,6
	500	4	12	8	8	4	0,8
	1000	11	17	14	14	3	1,4
	2500	6	3	1	3	3	0,3
	5000	11	13	8	11	3	1,1
	2AM(2)	136	103	120	120	17	12,0
TA 1537	0	6	5	7	6	1	-
	100	5	8	7	7	2	1,1
	500	11	4	11	9	4	1,4
	1000	10	7	5	7	3	1,2
	2500	5	10	3	6	4	1,0
	5000	7	8	7	7	1	1,2
	2AM(2)	158	185	126	156	30	26,1
TA 1538	0	25	27	28	27	2	-
	100	17	20	28	22	6	0,8
	500	22	27	30	26	4	1,0
	1000	33	21	32	29	7	1,1
	2500	21	35	36	31	8	1,2
	5000	36	29	29	31	4	1,2
	2AM(1)	1204	1139	1239	1194	51	44,8
TA 98 *	0	35	55	56	49	12	-
	100	52	59	49	53	5	1,1
	500	53	46	43	47	5	1,0
	1000	44	50	51	48	4	1,0
	2500	56	46	58	53	6	1,1
	5000	31	50	56	46	13	0,9
	2AM(1)	2593	2583	2681	2619	54	53,8
TA 100	0	83	70	45	66	19	-
	100	77	85	65	76	10	1,1
	500	61	81	60	67	12	1,0
	1000	67	62	64	64	3	1,0
	2500	72	60	73	68	7	1,0
	5000	87	69	67	74	11	1,1
	2AM(1)	2207	2004	2104	2105	102	31,9

0 : solvent experimental animal

proportion :  $\frac{\text{number of reverse mutants with product}}{\text{number of reverse mutants with solvent}}$

**5. BIBLIOGRAPHY**

1. Ames B.N., Lee F.D. and Durston W.E.  
An improved bacterial test system for the detection and classification of mutagens and carcinogens.  
Proc. Nat. Acad. Sci. USA, 70 (1973) 782-786.
2. Ames B.N., Mc Cann D. and Yamasaki E.  
Methods for detecting carcinogens and mutagens with the Salmonella Mammalian-microsome mutagenicity test.  
Mutation Res., 31 (1975), 347-364.
3. Ames B.N., Durston W.E., Yamasaki E. and Lee F.D.  
Carcinogens are mutagens : a simple test system combining liver homogenates for activation and bacteria for detection.  
Proc. Nat. Acad. Sci. USA, 70 (1973) 2281-2285.
4. Maron D.M. and Ames B.N.  
Revised methods for the Salmonella mutagenicity test.  
Mutation Res., 113 (1983), 173-215.

**6. ARCHIVES**

The following documents:

- the study protocol and possible amendments,
- all the original data,
- the correspondence,
- the study report (final) and possible amendments,

are kept in the archives of the Centre International de Toxicologie in Miserey, 27005 Evreux, France, for a period of 5 years after the end of the study. After this term, the study archives are, to the choice of the mandatory laboratory, either transferred to their quarters, or destroyed.

ANNEX

**ANALYSIS REPORT**

DESMODIUM Lot No 079

ANALYSES	RESULTS	NORMS
Visual research of other substances and moulds	absence	absence
<u>Identification</u>		
- Macroscopic aspect	conform	
- Microscopic aspect after colouring the transversal sections of leaves and stems	conform	identical to the samples already identified
<u>Doses</u>		
- Humidity percentage	9%	IO $\pm$ 2%
- Ash percentage	7,1%	7 $\pm$ 1%
- Indolic alkaloids proportion expressed in comparison with the tryptamine	4 mg/kg	< 25 $\mu$ g/l decoction 4 $\pm$ 0,4 mg/kg plants
- Organophosphors	not detected	absence
- Organochlores (Alpha Lindane) (Lindane)	20 mg/t 15 mg/t	20 mg/ton 20 mg/ton

# CIT

DESMODIUM ADSCENDENS:  
EVALUATION OF THE ACUTE TOXICITY  
ORALLY ADMINISTERED TO THE RAT

**CENTRE INTERNATIONAL DE TOXICOLOGIE**  
**(INTERNATIONAL TOXICOLOGY CENTRE)**

SEREY / BP 563 / 27005 ÉVREUX CEDEX FRANCE / TELEX 172046 F / FAX : +33 / 32 67 87 05

CONFIDENTIAL

DESMODIUM ADSCENDENS:  
EVALUATION OF THE ACUTE TOXICITY  
ORALLY ADMINISTERED TO THE RAT

**ADDRESSEE:**

Miss J. Ragot  
Centre de Recherches Phytothérapiques  
(Centre of Phytotherapeutic Research)  
23, Z.I. VIC  
Route d'Escalquens  
31320 Castanet-Tolosan

**DATE :**

October 18, 1989

**SUMMARY**

Title page	2
Summary	3
Attestation of the Study Director and other Scientist participating in this study	4

**CONCLUSION –UNITY OF QUALITY ASSURANCE** 5**SUMMARY** 6**1. INTRODUCTION** 7**2. MATERIALS AND METHODS** 8**2.1. Product** 8

## 2.1.1. Identification 8

## 2.1.2. Vehicle 8

## 2.1.3. Preparation 8

**2.2. Reactive system** 8

## 2.2.1. Animals 8

## 2.2.2. Environment 9

## 2.2.3. Food and drink 9

**2.3. Treatment** 10

## 2.3.1. Putting the animals on a hydrodiet 10

## 2.3.2. Product administration 10

## 2.3.3. Treatment date 10

**2.4. Clinical studies** 10

## 2.4.1. Clinical signs 10

## 2.4.2. Mortality 10

## 2.4.3. Corporal weight 11

**2.5. Pathology** 11**2.6. Archives** 11**3. RESULTS** 12**3.1. Clinical examinations** 12

## 3.1.1. Clinical signs 12

## 3.1.2. Mortality 12

## 3.1.3. Corporal weight 12

**3.2. Pathology** 12

Tables 1 to 3 13-15

Figure 1 16

**ANNEXES** 17**1. Information sheet and analysis report  
on the product to be tested** 18**2. Food formula** 21**3. Corporal weight of the control animals** 23-25

**ATTESTATION OF THE STUDY DIRECTOR**

This study was carried out in conformity with the protocol established with the 'Centre de Recherches Phytothérapiques' (Centre of Phytotherapeutic Research), the directive EEC 84/449/EEC annex V B<sub>1</sub> and following the rules of Good Laboratory Practice (France, Health Ministry, Instruction n° 1065, May 31, 1983).

I guarantee that this report is a true and sincere description of the applied procedures and of the results obtained in the execution of the study.

This study was carried out at the Centre International de Toxicologie (C.I.T.) in Miserey, 27005 Evreux, France.



Toxicology

-----  
J. Clouzeau      Date: October 18 1989  
Biologist

**OTHER SCIENTIST PARTICIPATING IN THIS STUDY**



Pharmacy  
Products preparation

-----  
M.H. Read      Date: October 18, 1989  
Biological pharmacist



## **SUMMARY AND CONCLUSION**

On demand of the Centre of Phytotherapeutic Research, the possible acute toxicity of the product **DESMODIUM ADSCENDENS** was evaluated on the rat, in conformity with the guideline n° 401 (OECD – February 24, 1987) for the control on products administered orally, and following the rules of Good Laboratory Practice (France, Health Ministry, Instruction n° 1065, May 31, 1983).

### **Material and Methods**

The product was administered to a group of 10 Sprague-Dawley rats (5 males and 5 females), put on a hydrodiet. The administration was effectuated with a decoction of the product in an aqueous solution in a dose of 100 mg/kg in a volume of 10 ml/kg. The decoction was prepared with the product in a concentration of 10 g/l water and brought to the boil during five minutes.

The mortality, the general conduct and the weight evolution of the animals were followed for a period of 14 days after the unique administration of the product. An anatomopathological exam was effectuated on each sacrificed animal at the end of the study.

### **Results**

There is no mortality at a dose of 100 mg/kg.

The general conduct and weight evolution of the animals are not influenced by the treatment.

The autopsy of the sacrificed animals at the end of the study has not demonstrated any macroscopic anomaly.

### **Conclusion**

Under our experimental conditions, not any clinical anomaly was observed after oral administration of a decoction of 100 mg/kg of the product **DESMODIUM ADSCENDENS** in the concentration of 10g/l water to the rat.

**1. INTRODUCTION**

Concerning the evaluation of toxic properties of a substance, the determination of the oral acute toxicity is usually an initial phase. It gives information on the health risks resulting from short-term exposition, by oral administration.

The substance to be tested is administered in a unique dose, orally, to a group of 10 rats.

## **2. MATERIAL AND METHODS**

### **2.1. PRODUCT**

#### **2.1.1. Identification**

The product **DESMODIUM ADSCENDENS**, supplied by the 'Centre de Recherches Phytothérapeutiques', 23 Z.I. Vic., Route d'Escalquens, 31320 Castanet-Tolosan, France, is presented in the form of a green leaf powder.

The product, conditioned in a glass phial of 2 g and in a plastic phial of 10 g, was received at the C.I.T. on 21.07.89, under the reference "DESMODIUM ADSCENDENS lot 079".

An information sheet and an analysis report from the 'Centre de Recherches Phytothérapeutiques' are presented in annex 1.

#### **2.1.2. Vehicle**

Water for injectable preparations lot n° 1271 (Biosédra, 92240 Malakoff, France).

#### **2.1.3. Preparation**

The product in the concentration of 10 g/l in water, was brought to a boil during 15 minutes.

## **2.2. REACTIVE SYSTEM**

### **2.2.1. Animals**

The animals used for this study, are rats of the strain ICO: OFA-SD (IOPS Caw), supplied by Iffa Crédo (69210 L'Arbresle, France).

At their arrival at the C.I.T., the animals were acclimatised in the experimentation space for a period of minimum 5 days, during which they were observed on a daily basis.

At the first day of their treatment, the animals are aged approximately 6 weeks and they have an average weight of  $171 \pm 6$  g for the males and of  $156 \pm 5$  g for the females. They are individually identified by perforation or notches at the auricle.

### 2.2.2. Environment

During the acclimatisation period and during the study, the animals are kept in an conventional air conditioned animal house. The maintenance conditions are as follows:

- ( Temperature :  $22 \pm 3$  °C
- ( Hygrometry :  $50 \pm 20$  % of relative humidity
- ( Dial : 12 hours of light / 12 hours of darkness

The non-recycled air is filtered by absolute filters.

The animals are harboured per 4 to 7 animals of the same sex during the acclimatisation period and per 5 of the same sex during the study. The animals are in a polycarbonate cage (dimensions 48 x 27 x 20 cm) able to be sterilized, equipped with a cover of stainless steel in the shape of a manger and with a feeding bottle of polypropylene (500 ml).

The animals sleep in sawdust litter that has been sifted and made dust-free (Société Parisienne des Sciures, 95100 Argenteuil, France). The analysis of potential residues and their main contaminants is carried out on a regular basis ('Laboratoire Municipal et Régional de Rouen' (Municipal and Regional Laboratory of Rouen) , 76000 Rouen, France).

### 2.2.3. Food and drink

The animals have at their disposal the nutrient "Rats and Mice maintenance reference A04 C" (U.A.R. 91360 Villemoisson-sur-Orge, France), in the shape of nibbles, administered ad libitum for the complete duration of the study (except for the fasting period at the moment of the treatment). The food analysis and research result of the main contaminants (pesticides, heavy metals, mycotoxins, etc...) are delivered by the supplier together with each lot. The formula of the nutrient can be found in annex 2.

The drinking water, filtered on 0,22 micron membrane (Société Millipore (Millipore Society), 78140 Vélizy, France) is administered ad libitum during the study. It is administered through feeding bottles. Bacteriological and chemical analyses and analyses of the main contaminants are carried out on a regular basis (Laboratoire Municipal et Régional de Rouen, 76000 Rouen, France).

The Study Director did not receive any information indicating the presence in the food or in the drinking water of non-food substances at such a level that it could interfere with the tested product.

## **2.3 TREATMENT**

### **2.3.1 Putting the animals on a hydrodiet**

On the eve of the treatment, the animals were put on a hydrodiet for some 18 hours before the product was administered. They were fed again 4 hours after the treatment.

### **2.3.2. Product administration**

A decoction of the product was administered in aqueous solution to the animals, in a dose of 100 mg/kg under a volume of 10 ml/kg. The administration is carried out in one time, orally, through an oesophageal tube with a round tip, in stainless steel (diameter 18 G.2" – Perfektum : Poffer & Sons Inc., New Hyde Park – New York 11040, USA).

### **2.3.3. Treatment date**

The sole administration was carried out on 23.08.89 in the morning (D 1). It was followed up for an observation period of 14 days until 06.09.89 (D 15).

## **2.4 CLINICAL STUDIES**

### **2.4.1 Clinical signs**

The observation of the animals is carried out on a regular basis in the hours after the administration of the product. It is carried out at least once a day in the following 14 days to register the clinical reversible or irreversible signs.

### **2.4.2 Mortality**

The mortality check is carried out immediately after administrating the product and at least 2 times a day during at least 14 observation days.

### 2.4.3 Corporal weight

The animals are weighed individually right before the product administration, then at D 5, D 8 and D 15. The weight evolution of the treated animals is compared with a reference curve of non-treated animals of the same initial weight, established at the C.I.T.

## **2.5 PATHOLOGY**

On the 15<sup>th</sup> day, the animals are sacrificed by making them inhale too much CO<sub>2</sub> and an autopsy is performed. After opening the abdominal and thoracic cavities, a macroscopic study of the main organs is carried out.

Because no injuries were found in the macroscopic study, no organs were removed and no histological study was performed.

## **2.6 ARCHIVES**

The following documents:

- the protocol and possible amendments,
- all the original data,
- the correspondence,
- the study report (final) and possible amendments,

are kept in the C.I.T. archives in Miserey, 27005 Evreux, France, for 5 hours after the end of the study in vivo. After this term, the study archives are, to the choice of the mandatory laboratory, either transferred to their quarters, or destroyed after agreement by the laboratory.

### **3. RESULTS**

#### **3.1. CLINICAL STUDIES**

##### **3.1.1 Clinical signs (table 1)**

Not any symptomatology was observed during the study.

##### **3.1.2 Mortality**

Not any mortality was observed during the observation period.

##### **3.1.3 Corporal weight** (table 2 and 3, figure 1) (experimental animals annex 3)

The weight evolution of the animals is normal.

#### **3.2. PATHOLOGY**

Not any apparent anomaly was detected in the macroscopic examination of the principal organs of the animals sacrificed at the end of the study.

Because no injuries were found in the macroscopic study, no organs were removed and no histological study was performed.

Table 1

Dose Mg/kg	Time	Animals		Symptomathology
		Males	Females	
100	15 min.	01-02-03-04-05	01-02-03-04-05	No symptomathology
	30 min.	01-02-03-04-05	01-02-03-04-05	No symptomathology
	1 hour	01-02-03-04-05	01-02-03-04-05	No symptomathology
	2 hours	01-02-03-04-05	01-02-03-04-05	No symptomathology
	4 hours	01-02-03-04-05	01-02-03-04-05	No symptomathology
	6 hours to D 15	01-02-03-04-05	01-02-03-04-05	No symptomathology

Table 2

dose mg/kg	volume ml/kg	sex	animals	Corporal weight (g)						
				D 1	(1)	D 5	(1)	D 8	(1)	D 15
100	10									
			01	178	49	227	22	249	67	316
			02	165	42	207	18	225	51	276
			03	170	48	218	22	240	54	294
		MALE	04	176	46	222	21	243	60	303
			05	166	45	211	21	232	54	286
			A	171	46	217	21	238	57	295
			SD	6	3	8	2	9	6	15
			01	162	28	190	7	197	11	208
			02	150	27	177	13	190	34	224
			03	158	32	190	7	197	26	223
		FE-	04	157	50	207	-5	202	25	227
		MALE	05	153	31	184	13	197	18	215
			A	156	34	190	7	197	23	219
			SD	5	9	11	7	4	9	8

(1) = Gain in weight  
A = Average  
SD = Standard Deviation

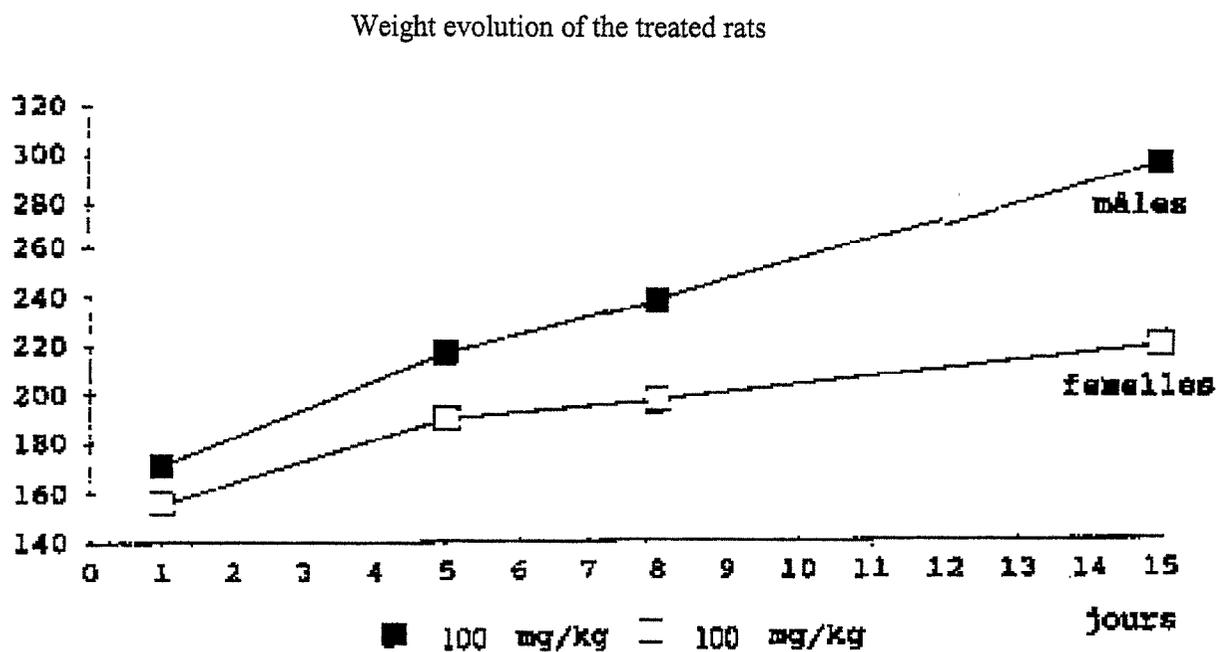
Table 3

Corporal weight gain (g)						
dose mg/kg	volume ml/kg	sex	D5-D1		D8-D5	D15-D8
100	10	male	A	46	21	57
			SD	3	2	6
100	10	female	A	34	7	23
			SD	9	7	9

A = Average

SD = Standard deviation

Figure 1



ANNEXES

1. INFORMATION SHEET AND ANALYSIS BULLETIN  
ON THE PRODUCT TO BE TESTED

**INFORMATION SHEET CONCERNING THE SUBSTANCE TO BE DOSED**

Laboratory name: Centre de Recherches Phytothérapeutiques

Name of the product: DESMODIUM ADSCENDENS

Chemical nomenclature: NON-BLOSSOMING AERIAL PARTS OF A PLANT OF THE  
PAPILLONACEA FAMILY

Developed formula:

State at 20°C and 760 mm Hg: SOLID

Solubility

- in the usual organic solvents: useful in aqueous decoction as herbal tea. Boil 15 mm, 10g of the plant in 1 litre of water.

Medium the product should be dosed in: workable doses for the decoction of the plant which contains among others indolic alkaloids

In which range of concentrations will it be?

<25µg/l decoction.

Do you know a dosing method in this medium?

- If you do, please let us know
- If you do not, do you know a dosing method of the product in another medium (please specify which)?:  
If you do, please let us know

Do you know the stability of the product? In dry powder more than 12 months

- If you do, please specify:       in which medium(s)  
  under which conservation condition(s)

Decoction is conserved at 3°C 24 hours a day.

Date: 19.07.89

Commissioner's signature: Jacqueline RAGOT

DESMODIUM Lot n° 079

<u>ANALYSES</u>	<u>RESULTS</u>	<u>NORMS</u>
Visual study of foreign substances and fungi	absence	absence
<u>Identification</u>		
Macroscopic aspect	conform	
Microscopic aspect after colouring the cross sections of the leaves and stems	conform	Identical to samples already identified
<u>Doses</u>		
Humidity percentage	9 %	IO ± 2%
Ash percentage	7,1 %	7 ± I%
Indolic alkaloids value in proportion to tryptamine	4 mg/kg	<25 µg/l decoction 4 ± 0,4 mg/kg plant
Organophosphorous	not detected	absence
Organochlorine (Alpha lindane ( Lindane	20 mg/t 15 mg/t	20 mg/ton 20 mg/ton

2. NUTRIENT FORMULA

Ref:  
**COMPLETE NUTRIENT  
 RATS – MICE MAINTENANCE**

PRESENTATION: NIBBLES OF Ø 15 mm  
 PACKAGING: SACK OF 25 kg

**Daily portion:** ACCORDING TO AGE AND WEIGHT, UNLIMITED WATER SUPPLY, RAT 18 to 25 g, MICE 8 to 12 g

**FORMULA %**

Cereals	88
Vegetable proteins (cattle cake, cellular proteins)	7
Animal proteins (fish)	2
Mineral vitaminized mix	3

**AVERAGE ANALYSIS %**

Caloric value (in Cal/kg)	2 900
Water	12
Proteins	17
Fats	3
Carbohydrates (N.F.E.)	58,7
Cellulose (Weende)	4,3
Minerals	5

**MINERALS**

(calculated in mg/kg)

	Nat. Suppl. (average)	Suppl. per CM	TOTAL
P	5 900	0	5 900
Ca	3 000	3 000	6 000
K	6 000	0	6 000
Na	400	1 600	2 000
Mg	2 000	130	2 130
Mn	40	40	80
Fe	90	150	240
Cu	15	15	30
Zn	40	45	85
Co	0,1	1,5	1,6

**AMINO ACIDS**

(calculated in mg/kg)

Arginine	9 800
Cystine	2 300
Lysine	8 500
Methionine	3 200
Tryptophane	1 900
Glycine	8 100

**VITAMINS**

(calculated in kg)

	Suppl. Nat. (average)	Synth. Suppl.	TOTAL
Vitam. A	1 000 IU	7 500 IU	8 500 IU
Vitam. D3	0 IU	1 500 IU	1 500 IU
Vitam. B1	6 mg	1 mg	7 mg
Vitam. B2	2 mg	4,50 mg	6,50 mg
Vitam. B3	10 mg	6,50 mg	16,50mg
Vitam. B6	1,4 mg	0,7 mg	2,1 mg
Vitam. B12	0,01 mg	0,01 mg	0,02 mg
Vitam. E	15 mg	15 mg	30 mg
Vitam. K3	0,25 mg	2,25 mg	2,50 mg
Vitam. PP	60 mg	15 mg	30 mg
Folic Ac.	0,5 mg	0 mg	0,5 mg
Biotin	0,04 mg	0 mg	0,04 mg
Choline	1 180 mg	400 mg	1 580mg

Available in quality "Contrôle" Ref. A04 C – A04 C R10

Rue Galliéni VILLEMOISSON 91360 ÉPINAY/ORGE Tél.: 69.04.03.57 Telex: UAR691716F

**3. CORPORAL WEIGHT OF THE TEST ANIMALS**

Corporal weight of the experimental rats  
(g)

dose mg/kg	volume ml/kg	sex		D1	D5	D8	D15
0	-	male	A	139	174	200	257
			SD	13	9	8	12
			n	15	15	15	15
0	-	female	A	126	158	173	194
			SD	5	7	6	11
			n	15	15	15	15

A = Average  
SD = Standard Deviation  
n = Animals

Corporal weight gain of the experimental rats  
(g)

dose mg/kg	volume ml/kg	sex		D5-D1	D8-D5	D15-D8
0	-	male	A	55	26	53
			SD	15	12	14
0	-	female	A	35	11	21
			SD	11	13	12

A = Average  
SD = Standard Deviation

Weight Evolution of the experimental rats

