

## CODING FORMS FOR SRC INDEXING

Microfiche No.		OTS0574128			
New Doc ID	88010000072	Old Doc ID	8EHQ-0201-14860		
Date Produced	12/14/00	Date Received	02/14/01	TSCA Section	8E
Submitting Organization		ATOFINA CHEMICALS INC			
Contractor		CTRE INTL DE TOXICOLOGIE			
Document Title		INITIAL SUBMISSION: SKIN SENSITIZATION TEST IN GUINEA PIGS (MAXIMIZATION METHOD OF MAGNUSSON AND KLIGMAN) OF FORAFAC 1203, WITH COVER LETTER DATED 2/9/2001			
Chemical Category		DIETHYLENE GLYCOL BUTYL ETHER, FLUORINATED COPOLYMER 71217,*			

A 03

BEHQ-0201-14850

JAH12



**ATOFINA**  
ATOFINA Chemicals, Inc.

RECEIVED  
OPPT/DCIC

2001 FEB 14 AM 6:58



BEHQ-01-14860

February 9, 2001

**UPS NEXT DAY DELIVERY**

Document Control Office (7407)  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
401 M Street, S.W.  
Washington, DC 20460  
Attn: 8(e) Submission

**Contain NO CBI**

Dear Sir/Madam:

ATOFINA Chemicals, Inc. (ATOFINA) is submitting a final report for a skin sensitization study in guinea pigs to the Environmental Protection Agency (EPA) pursuant to the Toxic Substances Control Act (TSCA) Section 8(e). The study does not involve effects in humans.

The enclosed study recently came into our possession via our parent company in France and provides information on a formulated product containing 40% of water (CASRN 7732-18-5), 30% of diethylene glycol butyl ether (CASRN 112-34-5), 11% of fluorinated copolymer with TSCA Inventory Accession number 71217, 8% of crotonic CYNA (CASRN 68877-55-4), 6% of 1-octanesulfonamide, N-(3-(dimethylamino)propyl)-5,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-, N-oxide (CASRN 80475-32-7), 4% of D-glucopyranose, oligomeric, decyl octyl glycosides (CASRN 68515-73-1), and 2% diethanolamine (CASRN 111-42-2).

The results of the study showed that in a guinea pig maximization test, a sensitization rate of 100% occurred.

Nothing in this letter or in the enclosed report is considered confidential business information of ATOFINA. Results from the study will be incorporated into the ATOFINA Material Safety Data Sheet for the material.

Further questions regarding this submission may be directed to me at (215) 419-5890.

Best Regards,

*Debra Randall*  
Debra Randall, DABT  
Product Safety Manager



88010000072

RECEIVED  
OPPT/DCIC  
2001 FEB 15 PM 12:02

ATOFINA Chemicals, Inc.  
2000 Market Street  
Philadelphia, PA 19103-3222  
215-419-7000  
www.AtofinaChemicals.com



**SPONSOR**  
Elf Atochem S.A  
Cours Michelet  
La Defense 10  
92091 Paris-la-Défense CEDEX  
France

RECEIVED  
CIT  
2001 FEB 14 11:06:55

**TEST SUBSTANCE**  
FORAFAC 1203

**STUDY TITLE**  
SKIN SENSITIZATION TEST  
IN GUINEA PIGS  
(Maximization method of Magnusson and Kligman)

**STUDY DIRECTOR**  
Xavier Manciaux

**STUDY COMPLETION DATE**  
14 December 2000

**TEST FACILITY**  
CIT  
Centre International de Toxicologie  
BP 563 - 27005 Evreux - France

**LABORATORY STUDY NUMBER**  
20023 TSG

**CONTENTS**

<b>STATEMENT OF THE STUDY DIRECTOR</b>	<b>4</b>
<b>OTHER SCIENTIST INVOLVED IN THIS STUDY</b>	<b>4</b>
<b>STATEMENT OF QUALITY ASSURANCE UNIT</b>	<b>5</b>
<b>SUMMARY</b>	<b>6</b>
<b>RESUME</b>	<b>8</b>
<b>1. INTRODUCTION</b>	<b>10</b>
<b>2. MATERIALS AND METHODS</b>	<b>10</b>
<b>2.1 TEST SUBSTANCE AND OTHER SUBSTANCES</b>	<b>10</b>
2.1.1 Identification of the test substance	10
2.1.2 Vehicle	10
2.1.3 Dosage form preparation	10
2.1.4 Other substances	11
<b>2.2 TEST SYSTEM</b>	<b>11</b>
2.2.1 Animals	11
2.2.2 Environmental conditions	11
2.2.3 Food and water	12
<b>2.3 TREATMENT</b>	<b>12</b>
2.3.1 Preliminary test	12
2.3.2 Main study	13
2.3.2.1 Preparation of the animals	13
2.3.2.2 Induction phase by intradermal and cutaneous routes	13
2.3.2.2.1 Intradermal route	13
2.3.2.2.2 Cutaneous route	13
2.3.2.3 Challenge phase	14
<b>2.4 SUMMARY DIAGRAM</b>	<b>15</b>
Figure 1: Treatment sites	15
<b>2.5 SCORING OF CUTANEOUS REACTIONS</b>	<b>16</b>
<b>2.6 CLINICAL EXAMINATIONS</b>	<b>16</b>
<b>2.7 BODY WEIGHT</b>	<b>16</b>
<b>2.8 PATHOLOGY</b>	<b>16</b>
2.8.1 Necropsy	16
2.8.2 Skin samples	16
2.8.3 Microscopic examination	16

CIT/Study No. 20023 TSG/FORAFAC 1203/Elf Atochem SA	3
2.9 DETERMINATION OF THE ALLERGENICITY LEVEL	16
2.10 CHRONOLOGY OF THE STUDY	17
2.11 PROTOCOL ADHERENCE	18
2.12 ARCHIVING	18
3. RESULTS	19
3.1 CHOICE OF THE VEHICLE	19
3.2 PRELIMINARY STUDY	19
3.2.1 Administration by intradermal route	19
3.2.2 Application by cutaneous route	20
3.3 MAIN STUDY	20
3.3.1 Clinical examinations	20
3.3.2 Body weight	20
3.3.3 Challenge phase - Scoring of cutaneous reactions	21
4. CONCLUSION	22
APPENDICES	23
1. Test article description and analytical certificate	24
2. Diet formula	27
3. Individual body weight values	29
4. Positive control to check the sensitivity of Dunkin-Hartley guinea pigs	31 and 32

**STATEMENT OF THE STUDY DIRECTOR**

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Décret N° 98-1312 du 31 décembre 1998 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 1er janvier 1999), Ministère de l'Economie, des Finances et de l'Industrie.
- Commission Directive 1999/11/EC of 8 March 1999 adapting to technical progress the Principles of Good Laboratory Practice as specified in Council Directive 87/18/EEC on the harmonization of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 77 of 23.3.1999).

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, Centre International de Toxicologie, BP 563, 27005 Evreux France.

Toxicology



X. Manciaux  
Study Director  
Doctor of Pharmacy

Date: 14 December 2000

**OTHER SCIENTIST INVOLVED IN THIS STUDY**

For Pharmacy: P.O. Guillaumat  
Doctor of Pharmacy

**STATEMENT OF QUALITY ASSURANCE UNIT**

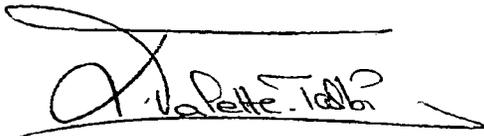
Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Protocol	22 March 2000	23 March 2000	23 March 2000
Report	22 November 2000	7 December 2000	7 December 2000

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.

The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and Principles of Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.



L. Valette-Talbi      Date: 14 December 2000  
Doctor of Biochemistry  
Head of Quality Assurance Unit

(\*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

## SUMMARY

At the request of Elf Atochem SA, Paris-la-Défense, France, the potential of the test substance FORAFAC 1203 (batch No. I-21/99) to induce delayed contact hypersensitivity was evaluated in guinea pigs according to the maximization method of Magnusson and Kligman and to OECD (No. 406, 17th July 1992) and EC (96/54/EEC, B.6, 30 July 1996) guidelines.

The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

## Methods

Thirty guinea pigs were allocated to two groups: a control group of five males and five females and a treated group of ten males and ten females.

On day 1, three pairs of intradermal injections were performed in the interscapular region of all animals:

- Freund's complete adjuvant (FCA) diluted at 50% (v/v) with 0.9% NaCl (both groups),
- test substance at the chosen concentration in the chosen vehicle (treated group) or vehicle alone (control group),
- test substance at the chosen concentration in a mixture FCA/0.9% NaCl 50/50 (treated group) or vehicle at the concentration of 50% (w/v) in a mixture FCA/0.9% NaCl 50/50 (control group).

On day 7, the same region received a topical application of sodium lauryl sulfate in vaseline (10%, w/w) in order to induce local irritation.

On day 8, the test substance (treated group) or the vehicle (control group) was applied topically to the same test site, which was then covered by an occlusive dressing for 48 hours.

On day 22, all animals of the treated and control groups were challenged by a cutaneous application of the test substance to the right flank. The left flank served as control and received the vehicle only. Test substance and vehicle were maintained under an occlusive dressing for 24 hours.

Skin reactions were evaluated approximately 24 and 48 hours after removal of the dressing.

Test substance concentrations were as follows:

### Induction (treated group)

- intradermal injections (day 1): FORAFAC 1203 at the concentration of 5% (w/w) in sterile isotonic saline solution (0.9% NaCl),
- topical application (day 8): FORAFAC 1203 undiluted.

### Challenge (all groups)

- topical application (day 22): FORAFAC 1203 undiluted.

At the end of the study, animals were killed without examination of internal organs.

Skin samples were taken from the challenge application sites of all the animals of treated group.

No histological examination was performed.

**Results**

No clinical signs and no deaths were noted during the study.

After the challenge application, no cutaneous reactions were observed in the animals of the control group.

In the treated group, cutaneous reactions attributable to delayed contact hypersensitivity (discrete or moderate erythema, sometimes associated with oedema) were observed in all animals.

**Conclusion**

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, the test substance FORAFAC 1203 (batch No. I-21/99) induces delayed contact hypersensitivity in 20/20 (100%) guinea pigs.

According to the classification criteria laid down in Directive 93/21/EEC (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/EEC, the test substance should be considered as a skin sensitizer.

## RESUME

A la demande de Elf Atochem SA, Paris-la-Défense, France, le potentiel du produit FORAFAC 1203 (lot n° I-21/99) à induire une hypersensibilisation cutanée retardée est évalué chez le Cobaye selon la méthode de maximisation de Magnusson et Kligman et conformément aux lignes directrices de l'OCDE (n° 406, 17 juillet 1992) et de la CEE (96/54/EEC, B.6, 30 juillet 1996).

L'étude est réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

## Méthodes

Trente cobayes sont répartis en 2 groupes : un groupe témoin de 5 mâles et 5 femelles et un groupe traité de 10 mâles et 10 femelles.

Au jour 1, 3 paires d'injections intradermiques sont effectuées au niveau de la région interscapulaire de tous les animaux :

- Adjuvant complet de Freund (FCA) dilué à 50 % (v/v) dans du NaCl à 0,9 % (groupe traité et groupe témoin),
- produit à tester à la concentration choisie dans le véhicule (groupe traité) ou véhicule seul (groupe témoin),
- produit à tester à la concentration choisie dans une mixture FCA/ NaCl à 0,9 % 50/50 (groupe traité) ou véhicule à la concentration de 50 % (p/v) dans une mixture FCA/ NaCl à 0,9 % 50/50 (groupe témoin).

Au jour 7, une application cutanée de laurylsulfate sodique à 10 % (p/p) dans de la vaseline est effectuée sur la même zone dans le but d'induire une irritation locale.

Au jour 8, le produit (groupe traité) ou le véhicule (groupe témoin) sont appliqués sur le même site, qui est ensuite recouvert d'un pansement occlusif pendant 48 heures.

Au jour 22, tous les animaux des groupes traité et témoin reçoivent une application cutanée déclenchante de produit sur le flanc droit. Le flanc gauche sert de témoin et reçoit le véhicule seul. Le produit et le véhicule sont maintenus sous pansement occlusif pendant 24 heures. L'évaluation des réactions cutanées est effectuée environ 24 et 48 heures après l'enlèvement du pansement.

Les concentrations de produit sont les suivantes :

### Induction (groupe traité)

- injections intradermiques (jour 1) : FORAFAC 1203 à la concentration de 5 % (p/p) dans du NaCl à 0,9 %,
- application cutanée (jour 8) : FORAFAC 1203 non dilué.

### Application déclenchante (tous les groupes)

- application cutanée (jour 22) : FORAFAC 1203 non dilué.

A la fin de l'étude, les animaux sont sacrifiés sans examen des organes internes.

Des prélèvements cutanés sont effectués au niveau des sites d'application déclenchante chez tous les animaux traités.

Aucun examen histologique n'est réalisé.

### Résultats

Aucun signe clinique ni aucune mortalité ne sont notés pendant l'étude.

Après l'application décienchante aucune réaction cutanée n'est observée chez les animaux du groupe témoin.

Dans le groupe traité, des réactions cutanées attribuables à une hypersensibilisation cutanée retardée (érythème discret ou modéré, parfois associé à un œdème) sont notées chez tous les animaux.

### Conclusion

Dans nos conditions expérimentales et selon la méthode de maximisation de Magnusson et Klügman, le produit FORAFAC 1203 (lot n° I-21/99) induit des réactions cutanées attribuables à une hypersensibilisation cutanée retardée chez 20/20 (100 %) Cobayes

Selon les critères de classification décrits dans la Directive 93/21/CEE (27 avril 1993) portant dix-huitième adaptation au progrès technique de la Directive 67/548/CEE, le produit est considéré sensibilisant par contact avec la peau.

## 1. INTRODUCTION

The objective of this study, performed according to the maximization method of Magnusson and Kligman (1), was to evaluate the potential of the test substance FORAFAC 1203 to induce delayed contact hypersensitivity in guinea pigs.

The results of the study are of value in predicting the contact sensitization potential of the test material in humans.

The study was conducted in compliance with:

- OECD guideline No. 406, 17th July 1992,
- EC Directive No. 96/54/EEC, B.6, 30 July 1996.

## 2. MATERIALS AND METHODS

### 2.1 TEST SUBSTANCE AND OTHER SUBSTANCES

#### 2.1.1 Identification of the test substance

The test substance FORAFAC 1203 used in the study was supplied by the Sponsor.

It was identified as follows:

- name:
  - protocol and labelling: FORAFAC 1203
- batch number:
  - protocol and labelling: I-21/99
- Elf Atochem filing number: CAL 4660/99
- description: brown liquid
- container: one plastic flask
- date of receipt: 24 March 2000
- storage conditions: at room temperature and protected from light
- composition: see analytical certificate
- expiry date: November 2000.

Data relating to the characterisation of the test substance are documented in a test article description and an analytical certificate (presented in appendix 1) provided by the Sponsor.

#### 2.1.2 Vehicle

The choice of the vehicle was based on tests to check the homogeneity (visual check) of the preparation (for cutaneous application and intradermal injections) and its free passage through a needle (for intradermal injections). The highest concentrations which satisfied these criteria were called the maximal practicable concentrations.

The vehicle used was 0.9% NaCl, batch Nos. LR92803 and 2934/1 (Laboratoire Frésenius, 92316 Sèvres, France).

#### 2.1.3 Dosage form preparation

All dosage form preparations were made freshly on the morning of administration and any unused material was discarded that same day.

---

(1) Magnusson B. and Kligman A.M.: The identification of contact allergens by animal assay. The guinea pig maximization test. *J. Invest. Derm.*, 52: 268-276 (1969).

#### 2.1.4 Other substances

The other substances used were Freund's complete adjuvant, batch Nos. 79H8938 and 119H8927 (Sigma, 38297 Saint-Quentin-Fallavier, France); sodium lauryl sulfate, batch No. 107H0006 (Sigma, 38297 Saint-Quentin-Fallavier, France) and vaseline, batch No. 1572 (Coopérative Pharmaceutique Française, 7000 Melun, France).

## 2.2 TEST SYSTEM

### 2.2.1 Animals

Species and sex: male and female guinea pigs.

Strain and sanitary status: Hartley CrI: (HA) BR, *Caesarian obtained, Barrier sustained - Virus Antibody Free (COBS - VAF®)*

Reason for this choice: species generally accepted by regulatory authorities for this type of study. The strain used has been shown to produce a satisfactory sensitization response using known sensitizers.

Breeder: Charles River France, 76410 Saint-Aubin-lès-Elbeuf, France.

Number: . one male and three females for the preliminary test,

. 30 animals (15 males and 15 females) for the main test.

Females were nulliparous and non-pregnant.

Allocation of the animals to the groups: on day -1, the animals were weighed and randomly allocated to two groups: a control group of ten animals (five males and five females) and a treated group of 20 animals (ten males and ten females).

Age/weight: on day 1, the animals of the main test were 1-3 months old and had a mean body weight  $\pm$  standard deviation of  $367 \pm 22$  g for the males and  $363 \pm 16$  g for the females.

Acclimation: at least 2 days before the beginning of the study.

Identification of the animals: ear-tattoo.

### 2.2.2 Environmental conditions

The conditions in the animal room were set as follows:

. temperature:  $21 \pm 2^\circ\text{C}$

. relative humidity: 30 to 70%

. light/dark cycle: 12 h/12 h

. ventilation: approximately 12 cycles/hour of filtered, non-recycled air.

The temperature and relative humidity were under continuous control and recording. The records were checked daily and filed. In addition to these daily checks, the housing conditions and corresponding instrumentation and equipment are verified and calibrated at regular intervals.

During the acclimation period and throughout the study, the animals were housed individually in polycarbonate cages (48 cm x 27 cm x 20 cm) equipped with a polypropylene bottle.

Dust-free sawdust was provided as litter (SICSA, 94142 Alfortville, France).

Bacteriological and chemical analyses of the sawdust, including the detection of possible contaminants (pesticides, heavy metals), are performed regularly by external laboratories.

The results of these analyses are archived at CIT.

### 2.2.3 Food and water

During the study, the animals had free access to "106 pelleted diet" (UAR, 91360 Villemoisson-sur-Orge, France).

Food is analysed regularly by the supplier for composition and contaminant levels.

The diet formula is presented in appendix 2.

Drinking water filtered by a FG Millipore membrane (0.22 micron) was provided *ad libitum*. Bacteriological and chemical analyses of the water and diet, including the detection of possible contaminants (pesticides, heavy metals and nitrosamines), are performed regularly by external laboratories.

The results of these analyses are archived at CIT.

No contaminants were known to have been present in the diet, drinking water or bedding material at levels which may be expected to have interfered with or prejudiced the outcome of the study.

## 2.3 TREATMENT

### 2.3.1 Preliminary test

A preliminary test was conducted in order to determine the concentrations to be tested in the main study.

By intradermal route (tested concentrations: 75%, 50%, 25%, 10%, 5% and 1% (w/w)):

- 24 hours before treatment, the dorsal region of the animals was clipped,
- intradermal administrations of the dosage form preparations (0.1 ml) were performed in the interscapular region,
- cutaneous reactions were evaluated approximately 24, 48 hours and 6 days after the injections.

By cutaneous route (tested concentrations: 100% and 50% (w/w)):

- 24 hours before treatment, both flank regions of the animals were clipped,
- the filter paper of a chamber (Finn Chamber<sup>®</sup>) was fully-loaded with the dosage form preparations. The chamber was then applied to the clipped area of the skin (one concentration per flank). The chamber was held in place by means of an occlusive dressing for 24 hours,
- cutaneous reactions were evaluated approximately 24 and 48 hours after removal of the dressings.

### Criteria for selection of concentrations

The following criteria were used:

- the concentrations should be well-tolerated systemically and locally,
- intradermal injections should cause moderate irritant effects (no necrosis or ulceration of the skin),
- cutaneous application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration,
- cutaneous application for the challenge phase should be the highest concentration which does not cause irritant effects.

### 2.3.2 Main study

#### 2.3.2.1 Preparation of the animals

For all animals, the application sites were:

- . clipped on days -1 and 7 (interscapular region 4 cm x 2 cm),
- . clipped and shaved on day 21 (each flank 2 cm x 2 cm),
- . clipped on day 25, before skin sampling, for the concerned animals (each flank 2 cm x 2 cm).

#### 2.3.2.2 Induction phase by intradermal and cutaneous routes

##### 2.3.2.2.1 Intradermal route

On day 1, six injections were made deep into the dermis of a 4 cm x 2 cm clipped interscapular area, using a needle (diameter: 0.50 x 16 mm) mounted on a 1 ml plastic syringe (0.01 ml graduations).

Three injections of 0.1 ml were made into each side of this interscapular region (i.e. three pairs of sites), as follows:

Injection	Site	Treated group	Control group
1	Anterior	FCA at 50% (v/v) in 0.9% NaCl	FCA at 50% (v/v) in 0.9% NaCl
2	Middle	test substance at 5% (w/w) in 0.9% NaCl	0.9% NaCl
3	Posterior*	test substance at 5% (w/w) in a mixture FCA /0.9% NaCl 50/50	vehicle at 50% (w/v) in a mixture FCA /0.9% NaCl 50/50

FCA: Freund's complete adjuvant

\* : The test substance was first dissolved in the aqueous phase prior to mixing with FCA. The final concentration of the test substance was equal to that used in injection 2.

The anterior and middle pairs of injections were performed close to each other and nearest the head, while the posterior pair was performed towards the caudal part of the test area.

##### 2.3.2.2.2 Cutaneous route

On day 7, the interscapular area was clipped.

As the test substance was shown to be non-irritant during the preliminary test, the animals were treated with 0.5 ml of sodium lauryl sulfate at the concentration of 10% (w/w) in vaseline, in order to induce local irritation.

On day 8, a pad of filter paper (approximately 8 cm<sup>2</sup>) was fully-loaded with the undiluted test substance and was then applied to the interscapular region of the animals of the treated group. The animals of the control group received an application of the vehicle alone under the same experimental conditions.

The pad was held in place for 48 hours by means of an adhesive hypoallergenic dressing and an adhesive anallergenic waterproof plaster.

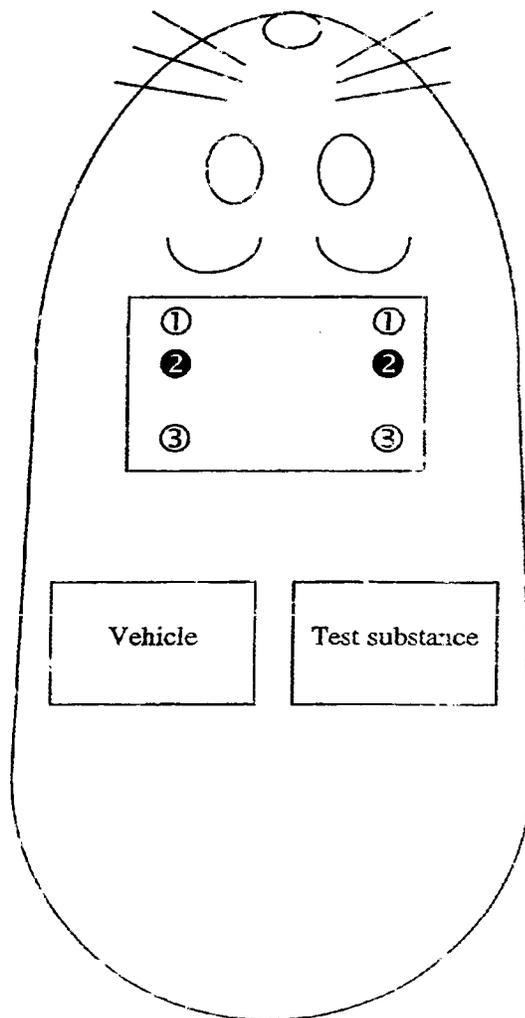
**2.3.2.3 Challenge phase**

On day 22, the animals of treated and control groups received an application of the test substance and vehicle. The filter paper of a chamber (Finn Chamber<sup>®</sup>) was fully-loaded with the undiluted test substance and was then applied to a clipped area of the skin of the posterior right flank of all animals.

The vehicle was applied under the same experimental conditions to the skin of the posterior left flank.

## 2.4 SUMMARY DIAGRAM

Figure 1: Treatment sites



### Induction site

Intradermal injections (day 1)\*

Cutaneous application (day 7):

Sodium lauryl sulfate 10% in vaseline

Cutaneous application (day 8):  
vehicle (control group)

or test substance at the chosen concentration (treated group)

### Challenge application sites

Cutaneous application (day 22)

#### \* Intradermal injections:

- ① 50% Freund's complete adjuvant and 0.9% NaCl
- ② vehicle (control group) or test substance at the chosen concentration in the vehicle (treated group)
- ③ vehicle at 50% (control group) or test substance at the chosen concentration (treated group) in the mixture Freund's complete adjuvant/0.9% NaCl (50/50)

**2.5 SCORING OF CUTANEOUS REACTIONS**

Twenty-four and 48 hours after removal of the dressing of the challenge application, both flanks of the treated and control animals were observed in order to evaluate cutaneous reactions, according to the following scale:

. no visible change .....	0
. discrete or patchy erythema .....	1
. moderate and confluent erythema.....	2
. intense erythema .....	3

Any observed oedema was recorded.  
Any other lesions were noted.

**2.6 CLINICAL EXAMINATIONS**

The animals were observed at least once a day during the study in order to check for clinical signs and mortality.

**2.7 BODY WEIGHT**

The animals were weighed individually on the day of allocation into the groups, on the first day of the study (day 1) and on the last day of the study (day 25).

**2.8 PATHOLOGY**

**2.8.1 Necropsy**

At the end of the study, all the animals were killed by carbon dioxide asphyxiation. No necropsy was performed.

**2.8.2 Skin samples**

At the end of the study, skin samples were taken from the posterior left and right flanks of all the animals showing skin reactions (all the animals of treated group).  
The samples were preserved in 10% buffered formalin.

**2.8.3 Microscopic examination**

No histological examination was performed.

**2.9 DETERMINATION OF THE ALLERGENICITY LEVEL**

The animals of the treated group show a positive reaction if macroscopic cutaneous reactions are clearly visible (score  $\geq 1$ ) and are of greater intensity and/or duration of response than the maximum reaction seen in control animals, or if macroscopic reactions are confirmed at microscopic examination as being due to the sensitization process.

Determination of the allergenicity level

The allergenicity level of the test substance is calculated by comparing the number of animals showing positive reactions with the number of surviving treated animals at the end of the study.

% of animals showing a reaction	Allergenicity level	Classification
0 - 8	I	weak
9 - 28	II	mild
29 - 64	III	moderate
65 - 80	IV	strong
81 - 100	V	extreme

According to the Commission Directive 93/21/EEC, when the reactions are positive in at least 30% of the treated animals, the test substance has sensitization properties and the symbol Xi, the indication of danger "Irritant" and the sentence "R 43: May cause sensitization by skin contact" must be applied.

The sensitivity of the experimental technique is regularly assessed using a known moderate sensitizer, MERCAPTOBENZOTHAZOLE. In a recent study performed under CIT experimental conditions, the strain of guinea pigs used showed a satisfactory sensitization response in 80% animals (see appendix 4).

## 2.10 CHRONOLOGY OF THE STUDY

The chronology of the main test is summarized as follows:

Procedure	Date	Day
Arrival of the animals	27 April and 3 May 2000	-9 and -2
Weighing and allocation of the animals into groups	4 May 2000	-1
Weighing, induction by intradermal injection	5 May 2000	1
Sodium lauryl sulfate application	11 May 2000	7
Induction by cutaneous route	12 May 2000	8
Removal of occlusive dressings	14 May 2000	10
Challenge cutaneous application	26 May 2000	22
Removal of occlusive dressings	27 May 2000	23
Scoring of cutaneous reactions after		
. 24 hours	28 May 2000	24
. 48 hours	29 May 2000	25
Weighing, sacrifice of the animals and skin samples	29 May 2000	25

## 2.11 PROTOCOL ADHERENCE

The study was performed in accordance with the Study Protocol No. 20023 TSG and subsequent amendments, with the following deviation from the agreed Study Protocol:

- . the acclimation period was reduced to 2 days for four animals of the control group.

This minor deviation was not considered to have compromised the validity or integrity of the study.

## 2.12 ARCHIVING

The study documentation and specimens generated during the course of the study are archived at CIT, 27005 Evreux, France, for 10 years after the end of the *in vivo* phase of the study.

The archived study materials include:

- . protocol and possible amendments,
- . raw data,
- . correspondence,
- . final report and possible amendments,
- . histological specimens:
  - tissues in preservative.

On completion of this period, the archived study materials will be returned to the Sponsor, or may be archived at CIT for a further period.

In addition, raw data not specific to the study including, but not limited to, certificates of analyses for food, water and bedding (if applicable) and records of environmental data and equipment calibration, are also archived at CIT and retained for at least 30 years.

### 3. RESULTS

#### 3.1 CHOICE OF THE VEHICLE

The vehicle chosen was 0.9% NaCl: a homogeneous dosage form preparation was obtained whatever the proportion.

The dosage form preparation at the concentration of 75% (w/w) passed freely through a needle and into the dermis.

#### 3.2 PRELIMINARY STUDY

##### 3.2.1 Administration by intradermal route

Results were as follows:

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment		
		24 hours	48 hours	6 days
male 301	75 + FCA	N	N	-
	75	N	N	-
	50 + FCA	N	N	-
	50	N	N	-
	25 + FCA	N	N	-
	25	N	N	-
female 302	75 + FCA	N	N	-
	75	N	N	-
	50 + FCA	N	N	-
	50	N	N	-
	25 + FCA	N	N	-
	25	N	N	-
female 303	10 + FCA	I	N	A
	10	I	N	A
	5 + FCA	I	I	I
	5	LI	LI	LI
	1 + FCA	I	I	I
	1	LI	LI	LI
female 304	10 + FCA	I	I	A
	10	I	N	A
	5 + FCA	I	I	I
	5	LI	LI	LI
	1 + FCA	I	I	I
	1	LI	LI	LI

FCA : mixture Freund's Complete Adjuvant/0.9% NaCl 50/50 (v/v)

N : necrosis

I : irritation

LI : slight irritation

A : crusts

- : not performed

In order to respect the criteria for the selection of concentrations (the concentration should be well-tolerated systemically and locally, intradermal injections should cause moderate irritant effect but no necrosis or ulceration of the skin), concentration chosen for the main study was 5% (w/w).

### 3.2.2 Application by cutaneous route

Results were as follows:

Animal number	Concentration of the test substance %		Scoring after removal of the dressing	
			24 hours	48 hours
male 301	100	RF	0	0
	50 (w/w)	LF	0	0
female 302	100	RF	0	0
	50 (w/w)	LF	0	0

RF: right flank

LF: left flank

On removal of the dressing, no residual test substance was observed.

In order to respect the criteria for the selection of concentrations (the concentrations should be well-tolerated systemically and locally, cutaneous application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration, cutaneous application for the challenge phase should be the highest concentration which does not cause irritant effect), concentration chosen for the topical application of the induction phase (day 8) and for the challenge application (day 22) was 100%.

## 3.3 MAIN STUDY

### 3.3.1 Clinical examinations

No clinical signs and no deaths were observed during the study.

### 3.3.2 Body weight

The body weight gain of the treated animals was similar to that of the control animals (appendix 3).

## 3.3.3 Challenge phase - Scoring of cutaneous reactions

On removal of the dressing, no residual test substance was observed.

Scoring of skin reactions was as follows:

		Control group					
Sex	Animal number	24 hours		48 hours			
		LF	RF	LF	RF		
Male	216	0	0	0	0		
	217	0	0	0	0		
	218	0	0	0	0		
	219	0	0	0	0		
	220	0	0	0	0		
Female	231	0	0	0	0		
	232	0	0	0	0		
	233	0	0	0	0		
	234	0	0	0	0		
	235	0	0	0	0		
		Treated group					
Sex	Animal number	24 hours		48 hours			
		LF	RF	LF	RF		
Male	221	0	2/Oe	0	2/Oe/S		
	222	0	2/Oe	0	2/Oe/S		
	223	0	1	0	1		
	224	0	2/Oe	0	2/Oe/S		
	225	0	2/Oe	0	2/Oe/S		
	226	0	1	0	2/S		
	227	0	1	0	1		
	228	0	2/Oe	0	1/S		
	229	0	2/Oe	0	2/Oe/S		
	230	0	2/Oe	0	2/Oe/S		
Female	236	0	2/Oe	0	2/Oe/S		
	237	0	2/Oe	0	1/S		
	238	0	1	0	1/S		
	239	0	1	0	1/S		
	240	0	1	0	1/S		
	241	0	2/Oe	0	2/Oe/S		
	242	0	2/Oe	0	2/Oe/S		
	243	0	1	0	2/Oe/S		
	244	0	2/Oe	0	1/S		
	245	0	2/Oe	0	LS		

LF : left flank (vehicle)

RF : right flank (undiluted test substance)

Oe : oedema

S : dryness of the skin

LS : scoring masked by a marked dryness of the skin

No cutaneous reactions were observed in the animals of the control group.

In the treated group, a discrete or moderate erythema (grade 1 or 2) was noted in all animals at the 24 and 48-hour readings. An oedema was recorded in 14/20 animals.

Dryness of the skin was observed in almost all animals at the 48-hour reading.

The observed cutaneous reactions were attributed to delayed contact hypersensitivity.

#### 4. CONCLUSION

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, the test substance FORAFAC 1203 (batch No. I-21/99) induces delayed contact hypersensitivity in 20/20 (100%) guinea pigs.

According to the classification criteria laid down in Directive 93/21/EEC (27th April 1993) adapting to technical progress for the eighteenth time Council Directive 67/548/EEC, the test substance should be considered as a skin sensitizer.

APPENDICES

**1. Test article description and analytical certificate**

TOXICOLOGY DEPARTMENT  
CONFIDENTIAL  
DTI 733  
14<sup>th</sup> March 2000

**elf atochem s.a.**

La défense 10, Cours Michelet  
92091 Paris-la-Défense cedex, France

**TEST ARTICLE DESCRIPTION**

**FORAFAC 1203**

**IDENTITY**

Test article name : FORAFAC 1203  
Origin : Elf Atochem Villiers-Saint-Paul  
Batch : I-21-99  
Elf Atochem filing number : CAL 4660/99

**PHYSICAL AND CHEMICAL PROPERTIES**

Appearance : Brown liquid  
Specific gravity : 1070 kg/m<sup>3</sup> at 20°C (liquid)  
Boiling point : 95°C  
Flash point : No flash point in the test conditions : (10 - 100°C)  
Solubility : Soluble in : Alcohols, Hydroxylated solvents.  
Insoluble in : Hydrocarbons, DMSO

**TOXICOLOGICAL INFORMATION AND USE SAFETY**

See Material and Safety Data Sheet

**STORAGE AND DISPOSAL**

Storage : in dark and at room temperature  
Expiry date : November 2000  
Disposal : incineration

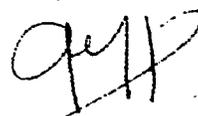
**elf atochem****ATO**

Villers Saint Paul, le 26 Novembre 1999

<b>CERTIFICAT D'ANALYSE FORAFAC 1203</b>
--

Lot N°	Unité	Résultat	Spécification de fabrication	Méthode d'analyse
I-21-99				
Extrait Scc	%	27,3	27 à 29	LCU 622
Ethanol	%	1,1	0 à 2,5	LCU 604
pH		8.6	8 à 9	LCU 642
Tension superficielle	Mn/m	15,9	0 à 16,5	LCU 663
Densité		1,083	1,06 à 1,09	LCU662
Foisonnement 0,5% dans eau de ville		9	6 à 50	LCU 670
Drainage 0,5% dans eau de ville	Minute	6	3 à 50	LCU 670
Pouvoir filmogène Sur heptane / eau de ville	Sec.	14	0 à 40	LCU 668

Le Chef de Service du Laboratoire  
SIGNATURE :



NOM : Robert Gruppo

2. Diet formula

Ref: 106  
**COMPLETE DIET**  
**GUINEA PIG MAINTENANCE DIET**  
 Appearance: 4.5 mm diameter granules  
 Conditioning: bags of 25 kgs

Daily portion: Guinea pigs 35-50 g, water *ad libitum*.

**FORMULA %**

Cereals .....	42
Grain byproducts and legumes..	46
Vegetable protein (soya bean meal, yeast) .....	9
Vitamin and mineral mixture....	3

**AVERAGE ANALYSIS %**

Calorific value (Kcal/kg) .....	2600
Moisture.....	10
Proteins .....	17
Lipids.....	3
Carbohydrates (N.F.E.) .....	49
Fibre.....	13
Minerals (ash) .....	8

MINERALS (calculated in mg/kg)			
	Nat. val.	CMV val.	Total
P.....	7400	1400	8800
Ca .....	5400	5600	11000
K .....	12000	0	12000
Na .....	1300	1950	3250
Mg .....	3270	130	3400
Mn .....	60	40	100
Fe.....	170	150	320
Cu .....	10	15	25
Zn .....	40	45	85
Co .....	0.1	1.5	1.6
I.....	0	0	0
Cl .....	0	0	0

**AMINO ACID VALUES**  
(calculated in mg/kg)

Arginine .....	8500
Cystine .....	2500
Lysine.....	7200
Methionine.....	2100
Tryptophan.....	2000
Glycine.....	6000

**FATTY ACID VALUES**  
(calculated in mg/kg)

Palmitic acid .....	3600
Palmitoleic acid .....	0
Stearic acid.....	700
Oleic acid .....	5900
Linoleic acid .....	11200
Linolenic acid .....	3000

VITAMINS (calculated per kg)			
	Nat. val.	CMV val.	Total
Vitamin A	3500 IU	7500 IU	11000 IU
Vitamin D3	30 IU	2000 IU	2030 IU
Vitamin B1	6 mg	6.4 mg	12.4 mg
Vitamin B2	5 mg	6.4 mg	11.4 mg
Vitamin B3	22 mg	26 mg	48 mg
Vitamin B6	0.7 mg	2.7 mg	3.4 mg
Vitamin B12	0.003 mg	0.012 mg	0.015 mg
Vitamin C	0 mg	400 mg	400 mg
Vitamin E	15 mg	60 mg	75 mg
Vitamin K3	5 mg	12.6 mg	17.6 mg
Vitamin PP	97 mg	14.5 mg	111.5 mg
Folic acid	2.2 mg	1.3 mg	3.5 mg
P.A.B. acid	0 mg	2.5 mg	2.5 mg
Biotin	0.02 mg	0.06 mg	0.08 mg
Choline	1010 mg	60 mg	1070 mg
Meso-Inositol	0 mg	62.5 mg	62.5 mg

This food is supplemented with stabilized coated vitamin C, avoiding the need of other food substances (greenery, ascorbic acid) if used within 4 months of date of manufacture.

3. Individual body weight values

## INDIVIDUAL BODY WEIGHT VALUES (g)

Groups	Sex	Animals	Days			
			-1	1	(1)	25
1	Male	216	334	334	192	526
		217	340	347	146	493
		218	358	368	156	524
		219	300	313	147	460
		220	375	392	99	491
		M	341	351	148	499
	SD	28	30	33	27	
	Female	231	385	391	90	481
		232	344	359	120	479
		233	353	370	63	433
		234	354	379	122	501
		235	360	369	111	480
		M	359	374	101	475
	SD	16	12	25	25	
	2	Male	221	367	367	84
222			352	364	135	499
223			351	366	177	543
224			349	357	160	517
225			377	386	120	506
226			379	388	138	526
227		364	373	187	560	
228		368	374	170	544	
229		392	392	122	514	
230		377	381	157	538	
M		368	375	145	520	
SD		14	12	31	31	
Female		236	352	358	26	384
		237	353	367	144	511
		238	371	376	97	473
	239	330	337	115	452	
	240	337	341	49	390	
	241	350	354	85	439	
242	331	338	113	451		
243	350	360	76	436		
244	360	376	128	504		
245	352	366	131	497		
M	349	357	96	454		
SD	13	15	38	44		

(1) = Body weight gain  
M = Mean  
SD = Standard Deviation

4. Positive control to check the sensitivity of Dunkin-Hartley guinea pigs

**Purpose: check the sensitivity of Dunkin-Hartley Guinea pigs (Breeder: Charles River France) to a positive control test article**

Method : Magnusson and Kligman  
 Test substance : MERCAPTOBENZOTHIAZOLE  
 CIT Study - Date : CIT/Study No. 19306 TSG - December 1999  
 Number of animals : one control group of 5 animals and one treated group of 10 animals  
 Induction : 1% (w/w) intradermal route day 1  
 20% (w/w) cutaneous route day 8  
 Challenge application: 20% (w/w) cutaneous route day 22

### Conclusion

Under our experimental conditions and according to the Magnusson and Kligman method, the test substance MERCAPTOBENZOTHIAZOLE at the concentration of 20% (w/w) induced positive skin sensitization reactions in 80% guinea pigs.

### INDIVIDUAL REACTIONS: CHALLENGE PHASE MACROSCOPIC FINDINGS

Groups	Sex	Animals	24-hour		48-hour		Conclusion
			LF	RF	LF	RF	
Control	Female	16	0	0/C	0	0/S/C	-
		17	0	0/C	0	0/S/C	-
		18	0	0/C	0	0/S/C	-
		19	0	0/C	0	0/S/C	-
		20	0	0/C	0	0/S/C	-
Treated	Female	21	0	2/C	1/S	2/C/S	+
		22	0	2/C	0	2/C/S	+
		23	0	2/C	0	2/C/S	+
		24	0	3/C	0	LS/C	+
		25	0	1/C	0	0/S	-
		26	0	2/C	0	LS/C	+
		27	0	3/C	0	LS/C	+
		28	0	1/C	0	LS/C	-
		29	0	2/C	0	LS/C	+
		30	0	2/C	0	0/S/C	+

LF : left flank (vehicle)

RF : right flank (test substance at the concentration of 20% (w/w))

S : dryness of the skin

LS : scoring masked by dryness of the skin

C : yellow coloration of the skin

- : negative

+ : hypersensitizing reactions