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December 18, 1995

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Attn: Section 8(e) Coordinator

**ORIGINAL**



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Subject: TSCA Section 8(e) Submission

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Dear Sir/Madam:

Elf Atochem North America Inc. is submitting the attached study to the Environmental Protection Agency (EPA) pursuant to Toxic Substances Control Act (TSCA) Section 8(e). This study provides information on dibenzyl disulfide (CAS No. 150-60-7) and does not involve effects in humans. The title of the enclosed study report is Dibenzyl disulfide Skin Sensitization Test in Guinea-Pigs (Maximization method of Magnusson, B. and Klingman, A.M.).



Nothing in this letter or the enclosed study report is considered confidential business information of Elf Atochem.

The following is a summary of the adverse effects observed in the skin sensitization test.

Dibenzyl disulfide was tested for potential to produce allergic skin reaction by intradermal injection and skin application to guinea pigs using a modified Magnusson and Klingman method. After challenge application, the test material produced evidence of a sensitization reaction in 100% (20/20) animals, and was classified as a sensitizer.

Elf Atochem has not previously filed any 8(e) notices or Premanufacture Notifications (PMNs) on the subject material.

Results from the study report will be incorporated into the current Elf Atochem Material Safety Data Sheet for dibenzyl disulfide.

Further questions regarding this submission may be directed to me at (215) 419-5892.

Sincerely,

C.H. Farr, PhD, DABT  
Manager, Product Stewardship  
and Toxicology



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Enclosure

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**SPONSOR**  
Elf Atochem Rotterdam B.V.  
P.O. Box 6030  
3196 XH Vondelingenplaat  
Netherland

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**STUDY TITLE**  
**SKIN SENSITIZATION TEST  
IN GUINEA-PIGS**  
(Maximization method of  
Magnusson, B. and Kligman, A.M.)

**TEST SUBSTANCE**  
**DIBENZYL DISULFIDE**

**STUDY DIRECTOR**  
Stéphane de Jouffrey

**STUDY COMPLETION DATE**  
15th November 1995

**PERFORMING LABORATORY**  
Centre International de Toxicologie (C.I.T.)  
Miserey - 27005 Evreux - France

**LABORATORY STUDY NUMBER**  
13043 TSG

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**STATEMENT OF THE STUDY DIRECTOR**

The study was performed in compliance with the following principles of Good Laboratory Practice Regulations:

- . O.E.C.D. principles of Good Laboratory Practice, C(81)30(final) Annex 2. May 12, 1981,
- . Décret N° 90-206 du 7 mars 1990 concernant les Bonnes Pratiques de Laboratoire (Ministère de l'Industrie et de l'Aménagement du Territoire).

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 the Centre International de Toxicologie (C.I.T.), Miserey, 27005 Evreux, France.

Toxicology



S. de Jouffrey      Date: 15.11.95  
Study Director  
Doctor of Veterinary Medicine  
Head of Short-term and Environmental  
Toxicology

**OTHER SCIENTISTS INVOLVED IN THIS STUDY**

For Pharmacy: J. Richard  
Doctor of Pharmacy

For Toxicology: C. Pelcot  
Study Supervisor

**STATEMENT OF QUALITY ASSURANCE UNIT**

## 1. Specific study inspections

Type of inspections	Dates (day/month/year)		
	Inspections	Report to Study Director (*)	Report to Management (*)
Protocol	23.5.95	31.5.95	31.5.95
Report	3.10.95	4.10.95	4.10.95

## 2. Routine inspections performed on other studies of the same type according to a frequency defined in Q.A.U. procedures

Inspected phase	Dates (day/month/year)		
	Inspections	Report to Study Director (*)	Report to Management (*)
Test substance/preparation	5.5.95	9.5.95	10.5.95
Treatment	21.4.95	24.4.95	24.4.95
Identification/housing	30.5.95	30.5.95	30.5.95

The inspections were performed in compliance with C.I.T. Quality Assurance Unit procedures and the Good Laboratory Practice Regulations.

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



L. Valette-Talbi    Date: 15.11.95  
 Doctor of Biochemistry  
 Head of Quality Assurance Unit  
 and Scientific Archives

## SUMMARY

At the request of Elf Atochem Rotterdam B.V., Vondelingenplaat, Netherland, the potential of the test substance DIBENZYL DISULFIDE to induce delayed contact hypersensitivity was evaluated in guinea-pigs according to the maximization method of Magnusson and Kligman and to O.E.C.D. (No. 406, 17th July 1992) and E.C. (92/69/E.E.C., B<sub>6</sub>) 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 1 (five males and five females) and a treated group 2 (ten males and ten females).

On day 1, in the dorsal region between the shoulders, intradermal injections of Freund's complete adjuvant mixed with the test substance (treated group) or the vehicle (control group) were prepared.

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

On day 8, this same test site was treated by topical application of the test substance (treated group) or the vehicle (control group) and was covered by an occlusive dressing for 48 hours.

Test substance and vehicle were maintained under an occlusive dressing for 24 hours. Skin reactions were evaluated approximately 24 and 48 hours later.

Test substance concentrations were as follows:

### Induction (treated group)

- . intradermal injections: DIBENZYL DISULFIDE at 1% (w/w) in paraffin oil,
- . topical application: DIBENZYL DISULFIDE at 20% (w/w) in paraffin oil.

### Challenge (all groups)

- . topical application: DIBENZYL DISULFIDE at 20% (w/w) in paraffin oil.

At the end of the study, animals were killed and cutaneous samples were taken from the challenge application sites from all the animals. No histological examinations were performed on the cutaneous reactions.

The sensitivity of the guinea-pigs in C.I.T. experimental conditions were checked in a recent study with a positive sensitizer: 2,4-dinitro-1-chlorobenzene. During induction period, the test substance was applied at 0.1% (day 1) and 5% (day 8) concentrations. At cutaneous challenge application, 1% (w/w) was tested on the right flank.

### **Results**

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

Slight to severe skin reactions (erythema grades 1 to 4, oedema grade 2 or 4, crusts, dryness of the skin) were observed at both readings in 20/20 treated animals. No skin reaction was noted in control animals.

The guinea-pigs which were used in a recent study, showed a satisfactory sensitization response in 95% animals using a positive sensitizer (appendix 5).

### **Conclusion**

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, cutaneous reactions attributable to the sensitization potential of the test substance DIBENZYL DISULFIDE were observed in 20/20 guinea-pigs.

## 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 DIBENZYL DISULFIDE 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 man.

The study was conducted in compliance with:

- . O.E.C.D. guideline No. 406, 17th July 1992,
- . E.C. Directive No. 92/69/E.E.C., B<sub>6</sub>, 31st July 1992.

## 2. MATERIALS AND METHODS

### 2.1. TEST AND CONTROL SUBSTANCES

#### 2.1.1 Test substance

The test substance, DIBENZYL DISULFIDE, used in the study was supplied by Elf Atochem.

Documentation supplied by the Sponsor identified the test substance as follows:

- . denomination:
  - protocol: DIBENZYL DISULFIDE
  - labelling: DIBENZYL DISULFIDE
- . batch number:
  - protocol: S.2708
  - labelling: S 2708
- . description: pink flakes
- . quantity and container: 200 g in a glass flask
- . date of receipt: 15.6.95
- . storage conditions: at room temperature and protected from light.

Data relating to the characterization 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 of the preparation (for topical and intradermal injections) and its free passage through a needle (for intradermal injections). The highest concentration which satisfied these criteria was called the maximal practicable concentration.

The vehicle used was paraffin oil, batch No. 7043 (Coopérative Pharmaceutique Française, 77000 Melun, France).

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

### 2.1.3 Preparation

The test substance was prepared at appropriate concentrations in the vehicle or Freund's complete adjuvant.

It was finely pulverised before being incorporated in the vehicle or adjuvant.

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

### 2.1.4 Other substances

The other substances used were sterile isotonic saline solution (0.9% NaCl), batch No. 4040 (Biosédra, 92240 Malakoff, France); Freund's complete adjuvant, batch No. 84H8800 (Sigma, 38297 Saint-Quentin-Fallavier, France); sodium laurylsulphate, batch No. 83H0841 (Sigma, 38297 Saint-Quentin-Fallavier, France) and vaseline, batch No. 4036 (Coopérative Pharmaceutique Française, 77000 Melun, France).

## 2.2. TEST SYSTEM

### 2.2.1 Animals

Species and strain: Dunkin-Hartley guinea-pigs.

Reason for this choice: species recommended by the international regulations for sensitization studies. The strain used has been shown to produce a satisfactory sensitization response using known positive sensitizers.

Breeder: Centre d'Élevage Lebeau, 78950 Gambais, France.

Number: 30 animals (15 males and 15 nulliparous and non-pregnant females).

Allocation of the animals to the groups: on day -1, the animals were weighed and randomly allocated to two groups: a control group 1 consisting of ten animals (five males and five females) and a treated group 2 consisting of 20 animals (ten males and ten females).

Weight: on day 1, the animals were approximately three months old and had a mean body weight (and standard deviation) of 363 (18) g for the males and 344 (28) g for the females.

Acclimatization: at least five days before the beginning of the study.

Identification of the animals: ear-tattoo.

### 2.2.2 Environmental conditions

During the acclimatization period and throughout the study, 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: about 12 cycles/hour of filtered, non-recycled air.

The temperature and relative humidity were recorded continuously and records retained.

The housing conditions (temperature, relative humidity, light/dark cycle and ventilation) were checked regularly.

During the acclimatization 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, 92142 Alfortville, France).

Bacteriological analysis of the sawdust and detection of possible contaminants (pesticides, heavy metals) are performed periodically.

### 2.2.3 Food and water

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

Each batch of food was analysed (composition and contaminants) by the supplier. The diet formula is presented in appendix 2.

Drinking water filtered by a F.G. Millipore membrane (0.22 micron) was provided *ad libitum*. Bacteriological and chemical analysis of the water and detection of possible contaminants (pesticides, heavy metals and nitrosamines) are performed periodically. Results are archived at C.I.T.

It was verified that no contaminants in the diet or water at levels likely to influence the outcome of the study were present.

## 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:

- . 24 hours before treatment, the dorsal region of the animals was clipped,
- . the test substance was prepared in an appropriate vehicle,
- . intradermal administrations of the test substance (0.1 ml) at different concentrations were performed in the dorsal region between the shoulders,
- . cutaneous reactions were evaluated approximately 24, 48 hours and seven days after injection.

#### By cutaneous route:

- . 24 hours before treatment, both flank regions of the animals were clipped,
- . if necessary, the test substance was prepared in an appropriate vehicle,
- . the test substance (0.5 ml for each concentration) was applied to a dry gauze pad of approximately 4 cm<sup>2</sup> which was held in place by 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 effect (no necrosis or ulceration of the skin),
- . topical application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration,
- . topical application for the challenge should be the highest concentration which does not cause irritant effect.

### 2.3.2 Main study

#### 2.3.2.1 Preparation of the animals

For all animals and before each treatment, the application sites were:

- . clipped on days -1 and 7 (scapular area 4 cm x 2 cm),
- . clipped and shaved on day 21 (each flank 2 cm x 2 cm),
- . clipped on day 25 (each flank 2 cm x 2 cm).

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

##### 2.3.3.1 Intradermal route

On day 1, six injections were made deep into the dermis of a clipped area (4 cm x 2 cm) in the dorsal region between the shoulders, using a needle (diameter: 0.50 x 16 mm, Térumo: C.M.L., 77140 Nemours, France) mounted on a 1 ml glass syringe (0.01 ml graduations, Record: Carrieri, 75005 Paris, France).

Three injections of 0.1 ml were made into each side of this shoulder region, as follows:

Injection sites*	Treated group	Control group
Anterior	1: FCA diluted at 50% (v/v) with 0.9% NaCl	1: FCA diluted at 50% (v/v) with 0.9% NaCl
Middle	2: test substance at 1% (w/w) in paraffin oil	2: vehicle
Posterior	mixture of 50/50 (w/v) of 1 and 2	mixture of 50/50 (w/v) of 1 and 2

\* : three pairs of sites

FCA: Freund's complete adjuvant

##### 2.3.3.2 Cutaneous route

On day 7, the scapular area was clipped. As the test substance was shown to be non-irritant during the preliminary tests, the animals were treated with 0.5 ml of sodium laurylsulphate (10% w/w) in vaseline in order to induce local irritation.

On day 8, a topical application to the region of the intradermal injections (4 cm x 2 cm) was performed.

##### Control group

- . application of 0.5 ml of the vehicle.

##### Treated group

- . application of 0.5 ml of the test substance at the chosen concentration.

The test substance and the vehicle were prepared on a dry gauze pad (Semmes France, 54183 Heillecourt, France), which was then applied to the dorsal region between the shoulders and held in place for 48 hours by means of an adhesive hypoallergenic dressing (Laboratoires de Pansements et d'Hygiène, 21300 Chenove, France) and an adhesive anallergenic waterproof plaster (Laboratoire des Professions Médicales, 92240 Malakoff, France).

On removal of the dressing, if present, any residual test substance was removed by means of a dry or a moistened gauze pad.

Cutaneous reactions were recorded one hour after removal of the occlusive dressing.

### 2.3.3.3 Challenge phase

On day 22, the animals from both groups received an application of 0.5 ml of the test substance at the chosen concentration to the posterior right flank, and 0.5 ml of the vehicle to the posterior left flank. This application was performed using a 1 ml plastic syringe (0.01 ml graduations, Térumo: C.M.L., 77140 Nemours, France). The test substance and vehicle were prepared on a dry gauze pad (Semes France, 54183 Heillecourt, France), then applied to a 4 cm<sup>2</sup> (2 cm x 2 cm) clipped area of the skin. The gauze pad was held in contact with the skin for 24 hours by means of an occlusive, hypoallergenic dressing (Laboratoires de Pansements et d'Hygiène, 21300 Chenove, France) and an adhesive anallergenic waterproof plaster (Laboratoire des Professions Médicales, 92240 Malakoff, France).

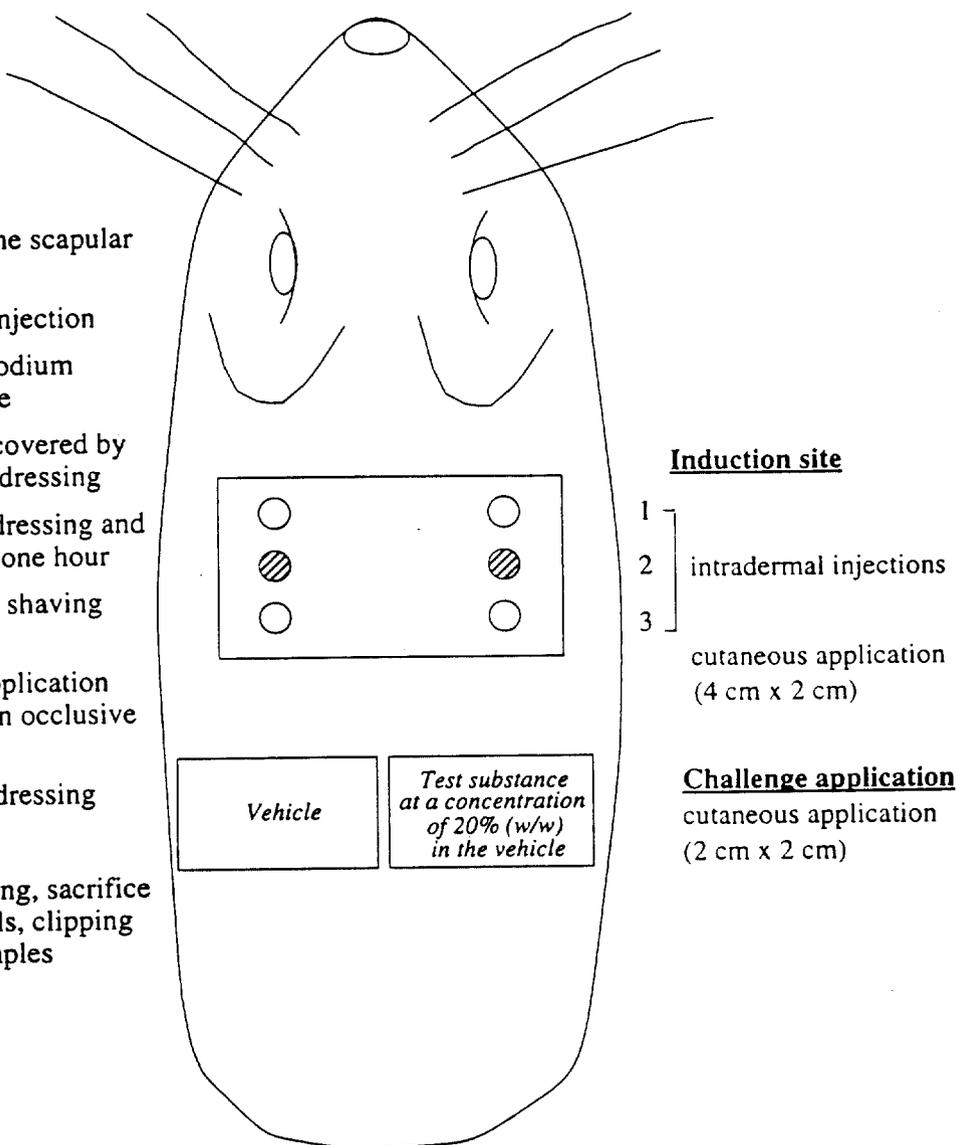
On removal of the dressing, if present, any residual test substance was removed by means of a dry or a moistened gauze pad.

## 2.4. SUMMARY DIAGRAMS

Figure 1: control group

## Chronology

- Day -1 Clipping of the scapular region
- Day 1 Intradermal injection
- Day 7 Clipping + Sodium laurylsulphate
- Day 8 Application covered by an occlusive dressing
- Day 10 Removal of dressing and scoring after one hour
- Day 21 Clipping and shaving of the flanks
- Day 22 Challenge application covered by an occlusive dressing
- Day 23 Removal of dressing
- Day 24 First scoring
- Day 25 Second scoring, sacrifice of the animals, clipping and skin samples

**Induction site**

- 1 } intradermal injections
- 2 } intradermal injections
- 3 } cutaneous application (4 cm x 2 cm)

**Challenge application**

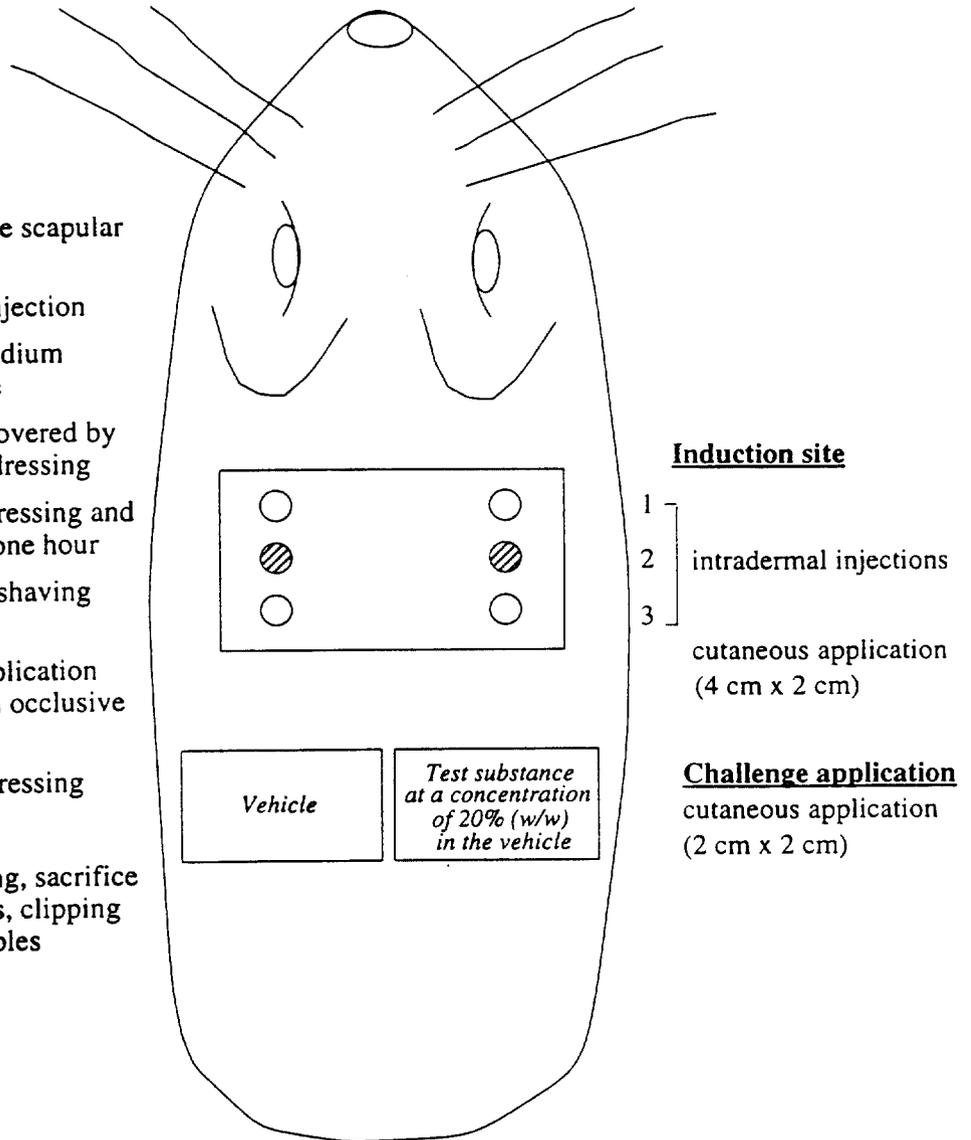
- cutaneous application (2 cm x 2 cm)

- Intradermal injections
- 1 } 50% Freund's complete adjuvant and sterile isotonic solution (0.9% NaCl)
- ▨ 2 } vehicle
- 3 } 1 + 2, 50/50 (w/v)

Figure 2: treated group

**Chronology**

- Day -1 Clipping of the scapular region
- Day 1 Intradermal injection
- Day 7 Clipping + Sodium laurylsulphate
- Day 8 Application covered by an occlusive dressing
- Day 10 Removal of dressing and scoring after one hour
- Day 21 Clipping and shaving of the flanks
- Day 22 Challenge application covered by an occlusive dressing
- Day 23 Removal of dressing
- Day 24 First scoring
- Day 25 Second scoring, sacrifice of the animals, clipping and skin samples



- Intradermal injections
- 1 } 50% Freund's complete adjuvant and sterile isotonic solution (0.9% NaCl)
- ◐ 2 } test substance at the chosen concentration
- 3 } 1 + 2, 50/50 (w/v)

## 2.5. SCORING OF CUTANEOUS REACTIONS

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

### Erythema and eschar formation

. No erythema.....	0
. Very slight erythema (barely perceptible).....	1
. Well-defined erythema .....	2
. Moderate to severe erythema .....	3
. Severe erythema (beet redness) to slight eschar formation (injuries in depth) .....	4

### Oedema formation

. No oedema.....	0
. Very slight oedema (barely perceptible).....	1
. Slight oedema (visible swelling with well-defined edges).....	2
. Moderate oedema (visible swelling raised more than 1 millimetre) .....	3
. Severe oedema (visible swelling raised more than 1 millimetre and extending beyond the area of exposure).....	4

Any other lesions were noted.

## 2.6. CLINICAL EXAMINATIONS

The animals were observed twice 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), on days 8 and 15 and on the last day of the study.

## 2.8. PATHOLOGY

### 2.8.1 Necropsy

At the end of the study, all the animals were killed by CO<sub>2</sub> inhalation in excess. No necropsy was performed.

### 2.8.2 Cutaneous samples

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

### 2.8.3 Microscopic examination

No histological examinations were performed.

## 2.9. DETERMINATION OF THE ALLERGENICITY LEVEL

The treated animals show a positive reaction if macroscopic cutaneous reactions are clearly visible (erythema  $\geq 2$ ) and if the treated animals have a greater intensity 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. Sensitization reactions are characterized at microscopic examination by basal spongiosis, reactional acanthosis of the epidermis and infiltration of mononucleated cells into the dermis (1).

### 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	very weak
9 - 28	II	weak
29 - 64	III	moderate
65 - 80	IV	strong
81 - 100	V	very strong

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

(1) Duprat, P. ; Delsaut, L. ; Gradiski, D. ; Lepage, M. : Investigations histopathologiques et cytologiques lors de la mise en évidence, chez le cobaye, d'une allergie cutanée de type retardé. *Revue Méd. Vét.* 127: 7, 1083-1101 (1976).

## 2.10. CHRONOLOGY OF THE STUDY

The chronology of the study is summarized as follows:

Procedure	Date	Day
Arrival of the animals	29.6.95	-8
Weighing and allocation of the animals into groups	6.7.95	-1
Weighing, induction by intradermal injection	7.7.95	1
Laurylsulfate application	13.7.95	7
Weighing, induction by cutaneous route	14.7.95	8
Removal of occlusive dressings and scoring of local reactions after one hour	16.7.95	10
Weighing	21.7.95	15
Challenge cutaneous application	28.7.95	22
Removal of occlusive dressings	29.7.95	23
Scoring of cutaneous reactions after . 24 hours	30.7.95	24
. 48 hours	31.7.95	25
Weighing, sacrifice of the animals and skin samples	31.7.95	25

## 2.11. ARCHIVES

The study documentation and materials, namely:

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

are stored in the archives of C.I.T., Miserey, 27005 Evreux, France, for five years after the end of the *in vivo* phase of the study. At the end of this period, the study documentation will be returned to the Sponsor.

### 3. RESULTS

#### 3.1. PRELIMINARY STUDY

##### 3.1.1 Administration by intradermal route

The maximal concentration which could pass through a needle as 1% (w/w). One test was performed in order to determine if this concentration was irritant.

Animal number	Concentration of the test substance % (w/w)	Scoring after treatment		
		24 hours	48 hours	7 days
male 01	1	irritation	irritation	irritation
female 01	1	irritation	irritation	irritation

Concentration chosen for the main study was 1% (w/w).

##### 3.1.2 Application by cutaneous route

The maximal practicable concentration was 20% (w/w). One test was performed in order to check if this concentration was irritant.

Animal number	Concentration of the test substance % (w/w)		Scoring after removal of the dressing (1)			
			24 hours		48 hours	
			E	O	E	O
male 01	20	RF	0	0	0	0
	20	LF	0	0	0	0
female 01	20	RF	0	0	0	0
	20	LF	0	0	0	0

E : erythema

O : oedema

RF: right flank

LF: left flank

(1): No residual test substance was observed.

Concentration chosen for the topical application of the induction phase (day 8) and for the challenge application was 20% (w/w).

### 3.2. MAIN STUDY

#### 3.2.1 Clinical examinations

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

The body weight gain of the treated animals was normal when compared to that of the control animals (figures 3 and 4, appendix 3).

#### 3.2.2 Scoring of cutaneous reactions

##### 3.2.2.1 End of the induction period

On day 10, after topical application of the induction period, signs of irritation were observed at the test site (dorsal region between shoulders) in the control and treated groups.

##### 3.2.2.2 Challenge application

Skin reactions were as follows:

Sex	Animal number	Control group							
		24 hours				48 hours			
		Erythema		Oedema		Erythema		Oedema	
		LF	RF	LF	RF	LF	RF	LF	RF
Male	61	0	0	0	0	0	0	0	0
	62	0	0	0	0	0	0	0	0
	63	0	0	0	0	0	0	0	0
	64	0	0	0	0	0	0	0	0
	65	0	0	0	0	0	0	0	0
Female	76	0	0	0	0	0	0	0	0
	77	0	0	0	0	0	0	0	0
	78	0	0	0	0	0	0	0	0
	79	0	0	0	0	0	0	0	0
	80	0	0	0	0	0	0	0	0

LF : left flank (control)

RF : right flank (treated)

Sex	Animal number	Treated group							
		24 hours				48 hours			
		Erythema		Oedema		Erythema		Oedema	
LF	RF	LF	RF	LF	RF	LF	RF		
Male	66	0	3/S	0	2	0	3/S	0	0
	67	0	2	0	0	0	1/S	0	0
	68	0	2	0	0	0	1/S	0	0
	69	0	3/S	0	2	0	3/S	0	0
	70	0	3/A	0	2	0	2/S/A	0	0
	71	0	3	0	4	0	2/S	0	0
	72	0	3/S	0	2	0	2/S	0	0
	73	0	1	0	0	0	1/S	0	0
	74	0	3/S	0	4	0	2/S	0	0
	75	0	4	0	4	0	4/N/S	0	0
Female	81	0	2/S	0	0	0	2/S	0	0
	82	0	3/S	0	2	0	3/S	0	0
	83	0	3	0	2	0	2/S	0	0
	84	0	3/S	0	4	0	3/S	0	0
	85	0	3/S	0	4	0	2/S	0	0
	86	0	2/S	0	0	0	1/S	0	0
	87	0	3	0	4	0	2/S	0	0
	88	0	2	0	0	0	1/S	0	0
	89	0	3	0	0	0	2/S	0	0
	90	0	2	0	2	0	2/S	0	0

LF: left flank (control)

RF: right flank (treated)

S : dryness of the skin

A : crust

N : necrosis

No residual test substance was observed after removal of the dressing.

In the absence of effect in the control group, these cutaneous reactions were attributed to a sensitization effect of the test substance.

### 3.2.3 Pathology

#### 3.2.3.1 Microscopic examination

No microscopic examinations were performed.

## 4. CONCLUSION

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, cutaneous reactions attributable to the sensitization potential of the test substance DIBENZYL DISULFIDE were observed in 20/20 guinea-pigs.

Figure 3: Male body weight (g)

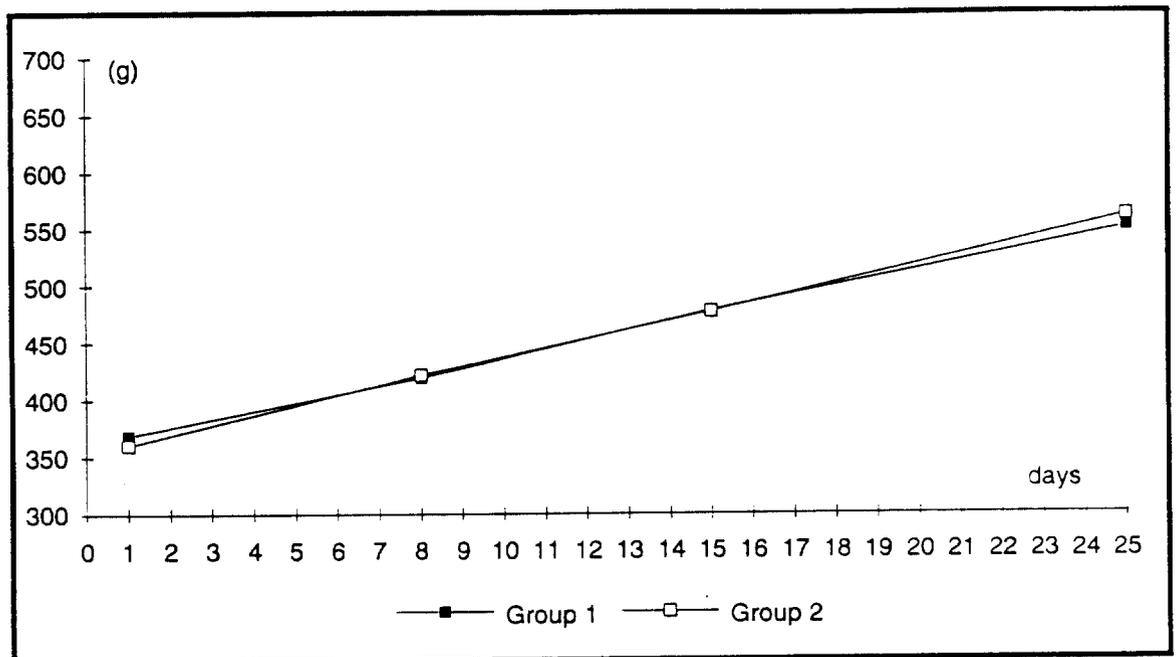
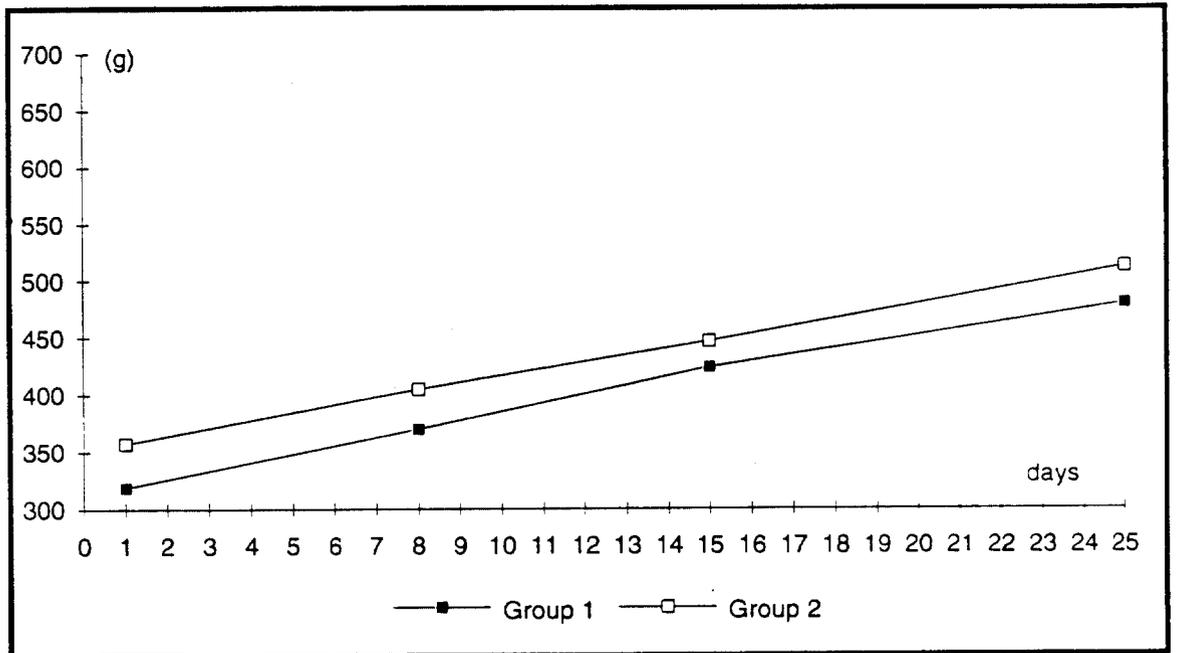


Figure 4: Female body weight (g)



**APPENDICES**

1. Test article description and analytical certificate

TOXICOLOGY DEPARTMENT  
CONFIDENTIAL  
17 May 1995

**elf atochem s.a.**

La défense 10, cedex 42  
92091 Paris-la-Défense, France

*TEST ARTICLE DESCRIPTION*

**DIBENZYL DISULFIDE**

**IDENTITY**

Test article name : Dibenzyl disulfide  
Chemical name : Disulfide, bis(phenylmethyl)  
CAS number : 150-60-7  
EINECS number : 2057640  
Molecular formula : C<sub>14</sub>H<sub>14</sub>S<sub>2</sub>  
Molecular weight : 246  
Origin and batch : Elf Atochem UK; S 2708  
Elf Atochem filing number : CAL 2413/95

**PHYSICAL AND CHEMICAL PROPERTIES**

Appearance : pink flakes  
Specific gravity : 1.30 at 20°C  
Melting point : 69°C  
Boiling point : 210-216°C at 24 mbar  
Flash point : 150°C (closed cup)  
Solubility : insoluble in water  
: 10% in oil at 25°C

**TOXICOLOGICAL INFORMATION AND USE SAFETY**

See "Fiche de Données de sécurité".

**STORAGE AND DISPOSAL**

Storage : in dark and at room temperature  
Expiry date : December 1996  
Disposal : incineration

**elf atochem**

Elf Atochem UK Ltd  
 Chlorobluene Derivatives Division  
 West Bank Dook Estate, Widnes, Cheshire WA8 0NY  
 Tel: 051 424 4281 Sales Fax: 051 423 2814 Tlx: 628470  
 Works Fax: 051 423 6757 Tlx: 628244

### CERTIFICATE OF ANALYSIS

Customer	ELF ATOCHEM, LEVALLOIS	
Quantity	1 X 0.2 Kg	Date 23 - 03 - 95
Product	DIBENZYL DISULPHIDE	
Customers Order No.		Order No. S 2708

The product supplied against the above order has been tested and found to comply with the requirements of

Batch Numbers etc.	Analysis Results
--------------------	------------------

COPPER COROSION : 1A

MELTING POINT

FIRST MENISCUS : 65.6° C

COMPLETELY MELTED : 69.0° C

\_\_\_\_\_  
 Quality Control Manager  
 (J. Jolley)

The product supplied against the above order, whilst meeting Elf Atochem's published sales specification, has been manufactured outside the scope of the Atochem UK (Widnes) BS5750 / ISO 9002 accreditation.

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 biproducts 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

**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

**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

**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.

U.A.R., 7 rue Galliéni, 91360 Villemoisson - Tel: 69.04.03.57 - Fax : 69.04.81.97  
(Ref. Doc. UAR: 1992)

### 3. Individual body weight values

**INDIVIDUAL BODY WEIGHT VALUES**  
(g)

Groups	Sex	Animals	Days								
			-1	1	(1)	8	(1)	15	(1)	25	
1	Male	61	379	364	8	372	93	465	58	523	
		62	359	352	64	416	48	464	80	544	
		63	382	377	83	460	53	513	111	624	
		64	365	366	48	414	66	480	52	532	
		65	396	385	47	432	36	468	63	531	
		M	376	369	50	419	59	478	73	551	
		SD	15	13	28	32	22	21	24	42	
		Female	76	341	328	47	375	62	437	82	519
	77		333	314	31	345	64	409	53	462	
	78		320	314	69	383	49	432	57	489	
	79		327	316	62	378	40	418	59	477	
	80		332	321	46	367	54	421	30	451	
	M		331	319	51	370	54	423	56	480	
	SD		8	6	15	15	10	11	19	26	
	2		Male	66	381	371	79	450	46	496	93
		67		342	317	64	381	62	443	80	523
68		356		363	46	409	61	470	80	550	
69		359		356	78	434	54	488	66	554	
70		353		357	60	417	26	443	91	534	
71		343		340	68	408	58	466	109	575	
72		376		383	64	447	51	498	109	607	
73		392		388	62	450	69	519	86	605	
74		367		359	59	418	53	471	64	535	
75		376		372	29	401	75	476	63	539	
M		365	361	61	422	56	477	84	561		
SD		17	21	15	23	13	24	17	31		
Female		81	397	388	18	406	40	446	59	505	
		82	393	392	45	437	52	489	59	548	
		83	306	321	60	381	44	425	59	484	
		84	345	341	51	392	34	426	81	507	
		85	317	333	41	374	35	409	56	465	
		86	348	357	47	404	26	430	62	492	
		87	354	356	38	394	58	452	57	509	
		88	362	358	55	413	48	461	72	533	
	89	336	339	58	397	47	444	81	525		
	90	374	388	60	448	34	482	66	548		
M	353	357	47	405	42	446	65	512			
SD	30	25	13	23	10	26	10	27			

(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 (Centre d'élevage Lebeau) to a positive control test article**

Method : Magnusson and Kligman  
 Test substance : 2,4-dinitro-1-chlorobenzene  
 C.I.T. Study - Date : December 1994 (CIT/Study No. 12437 TSG)  
 Number of animals : 20 females  
 Induction : 0.1% intradermal route day 1  
 5% cutaneous route day 8  
 Challenge application: 1% right flank  
 paraffin oil left flank

### Conclusion

Under our experimental conditions and according to the Magnusson and Kligman method, 2,4-dinitro-1-chlorobenzene at a concentration of 1% (w/w) induced positive skin sensitization reactions in 95% of the guinea-pigs.

### INDIVIDUAL REACTIONS: CHALLENGE PHASE MACROSCOPIC FINDINGS

Group	Sex	Animals	24-hour				48-hour				Conclusion	
			Erythema		Oedema		Erythema		Oedema		LF	RF
			LF	RF	LF	RF	LF	RF	LF	RF	LF	RF
Treated	Female	11	0	2	0	0	0	2/S	0	0	-	+
		12	0	2	0	0	0	2/S/A	0	0	-	+
		13	0	1	0	0	0	1/S	0	0	-	+/-
		14	0	3	0	2	0	3/S	0	0	-	+
		15	0	2	0	2	0	2/S	0	0	-	+
		16	0	4	0	2	0	4	0	0	-	+
		17	0	3	0	2	0	1/S	0	0	-	+
		18	0	2	0	0	0	2/S	0	0	-	+
		19	0	2	0	0	0	2/S	0	0	-	+
		20	0	3	0	2	0	3/S	0	2	-	+
		21	0	3	0	2	0	3/S	0	0	-	+
		22	0	3	0	2	0	3/S	0	0	-	+
		23	0	2	0	0	0	1/S	0	0	-	+
		24	0	3	0	2	0	2/S	0	0	-	+
		25	0	2	0	0	0	2/S	0	0	-	+
		26	0	3	0	2	0	2	0	0	-	+
		27	0	2	0	0	0	1/S	0	0	-	+
		28	0	3	0	2	0	2	0	0	-	+
		29	0	3	0	2	0	3/S	0	2	-	+
		30	0	3	0	2	0	3/S	0	2	-	+

- : negative

+ : hypersensitizing reactions

+/- : borderline reactions

S : dryness of the skin

A : crust

LF: left flank

RF: right flank

**Triage of 8(e) Submissions**

Date sent to triage: 5/28/96

**NON-CAP**

**CAP**

Submission number: 13572A

TSCA Inventory: **(Y)** N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO            AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX            SBTOX            ~~SEN~~            w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX            CTOX            EPI            RTOX            GTOX  
STOX/ONCO    CTOX/ONCO    IMMUNO        CYTO            NEUR

Other (FATE, EXPO, MET, etc.): \_\_\_\_\_

Notes:

**THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY**

**For Contractor Use Only**

entire document: **(0)** 1 2 pages 1 pages \_\_\_\_\_

Notes:

Contractor reviewer : JW Date: 3/29/96



CECATS/TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA: Submission # BEHO 0196-13572 SEQ. A  
 TYPE: (INT) SUPP FLWP  
 SUBMITTER NAME: ELF ATOCHEM  
NORTH AMERICA, INC.

INFORMATION REQUESTED: FLWP DATE  
 0501 NO INFO REQUESTED  
 0502 INFO REQUESTED (TECH)  
 0503 INFO REQUESTED (VOL ACTIONS)  
 0504 INFO REQUESTED (REPORTING RATIONALE)  
 DISPOSITION:  
(0505) REFER TO CHEMICAL SCREENING  
 0578 CAP NOTICE

VOLUNTARY ACTIONS:  
 0401 NO ACTION REQUIRED  
 0402 STUDIES PLANNED/IN PROGRESS  
 0403 MODIFICATION OF WORK PLAN  
0404 LABEL/MSDS CHANGES  
 0405 PROCESS/ANALYSIS CHANGES  
 0406 APP/USE DISCONTINUED  
 0407 PRODUCTION DISCONTINUED  
 0408 CONFIDENTIAL

SUB. DATE: 12/18/95 OIS DATE: 01/16/96 CSRAD DATE: 02/15/96

CHEMICAL NAME: Misc. Chemicals CAS# 150-60-7  
None

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
0201 ONCO (HUMAN)	01 02 04	EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL. TRANS (IN VITRO)	01 02 04	HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEMPHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	ECO/AQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	ENV. OCCUREL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	RESPONSE REQEST DELAY	01 02 04	0248 PROD/USE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	PROD/COMP/CHEM ID	01 02 04	0251 MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	REPORTING RATIONALE	01 02 04	0299 OTHER	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX. (ANIMAL)	01 02 04	ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX. (ANIMAL)	01 02 04	METAB/PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX. (ANIMAL)	01 02 04	METAB/PHARMACO (HUMAN)	01 02 04		

TRIAGE DATA: NON-CBI INVENTORY YES (YES) NO  
ONGOING REVIEW YES (DROP/REFER) NO (CONTINUE) REFER  
 SPECIES GP TOXICOLOGICAL CONCERN: LOW MED HIGH  
 USE: PRODUCTION:

UNCLASSIFIED

13572A

H

Dermal sensitization in guinea pigs is of high concern. Skin sensitization potential was evaluated in 20 guinea pigs using the Magnusson and Kligman method. After the challenge application, 20/20 animals (100%) exhibited positive reactions.