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September 25, 1996

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Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M St., S.W.
Washington, D.C. 20460

Subject: TSCA Section 8(e) Submission

8EHQ-1096-13751

Dear Sir/Madam:

Elf Atochem North America Inc. (Elf Atochem) is submitting the attached study to the Environmental Protection Agency (EPA) pursuant to the Toxic Substances Control Act (TSCA) Section 8(e). This study provides information on di(2-ethylhexyl)peroxydicarbonate (CAS No. 16111-62-9) and does not involve effects in humans. The title of the study is Skin Sensitization Test in Guinea-Pigs (Maximization Method of Magnusson, B. And Kligman, A.M.).

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

The results of the study showed the test material was a skin sensitizer in guinea pigs. Results from the study will be incorporated into the Elf Atochem Material Safety Data Sheet for the material.

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

Sincerely,

Debra Randall, DABT
Product Safety Manager



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SPONSOR

Elf Atochem Deutschland
Luperox Division
Denzinger Strasse Postfach 1354
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Germany

STUDY TITLE

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

TEST SUBSTANCE

LUPEROX 223-M-75
(DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE)
CAS Registry Number 16111-62-9

STUDY DIRECTOR

Stéphane de Jouffrey

STUDY COMPLETION DATE

29 August 1996

PERFORMING LABORATORY

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

LABORATORY STUDY NUMBER

14052 TSG

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STATEMENT OF THE STUDY DIRECTOR

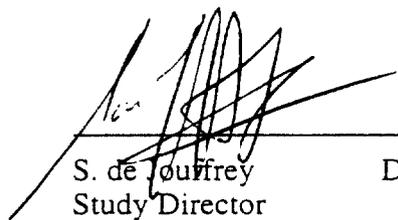
The study was performed in compliance with the following principles of Good Laboratory Practice Regulations:

- . 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
Study Director

Date: 29 August 1996

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	3 May 96	27 June 96	27 June 96
Report	22 July 96	22 July 96	24 July 96

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	23 Feb. 96	26 Feb. 96	26 Feb. 96
Treatment/test substance	16 Feb. 96	19 Feb. 96	19 Feb. 96
Animals/housing	12 Mar. 96	20 Mar. 96	27 Mar. 96

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: 29 August 1996
Doctor of Biochemistry
Head of Quality Assurance Unit
and Scientific Archives

SUMMARY

At the request of Elf Atochem Deutschland, Günzburg, Germany, the potential of the test substance LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) (batch No. 802-9409-01) 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., B₆) guidelines. The solvent of the test substance, Isododecan, was also tested under the same experimental conditions. The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

Forty guinea-pigs were allocated to three groups: two control groups 1 and 2 (five males and five females each) and a treated group 3 (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), paraffin oil (control group 1) or Isododecan (control group 2) 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), paraffin oil (control group 1) or Isododecan (control group 2), and was covered by an occlusive dressing for 48 hours.

After a rest period of 12 days, the animals of the control group 1 were challenged by a topical application of the test substance to the right flank and Isododecan to the left flank; the animals of the control group 2 were challenged by Isododecan to the right and the left flanks; the animals of the treated group 3 were challenged by the test substance to the right and the left flanks.

The products were maintained under an occlusive dressing for 24 hours. Skin reactions were evaluated approximately 24, 48 and 72 hours later.

Product concentrations were as follows:

Control group 1

Induction

- . intradermal application: paraffin oil
- . topical application: paraffin oil.

Challenge

- . topical application:
 - LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) undiluted (left flank)
 - Isododecan undiluted (right flank).

Control group 2

Induction

- . intradermal application: Isododecan at 50% (w/w) in paraffin oil
- . topical application: Isododecan undiluted.

Challenge

- topical application: Isododecan at 50% (w/w) in paraffin oil (left flank)
Isododecan undiluted (right flank).

Treated group 3

Induction

- intradermal injections:
LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) at 2.5% (w/w) in a mixture paraffin oil/Isododecan
- topical application:
LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) undiluted.

Challenge

- topical application:
LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) at 50% (w/w) in Isododecan (left flank)
LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) undiluted (right flank).

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.

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 chlorobenzene. During induction period, the test substance was applied at 0.1% (day 1) and 1% (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.

In the control groups 1 and 2, no cutaneous reactions were observed 24, 48 and 72 hours after removal of the dressings of the challenge cutaneous application. Only dryness of the skin was noted in one animal from group 1 at each reading, and in almost all animals from both groups at the 72-hour reading.

In the treated group, marked cutaneous reactions, similar on both flanks, were observed. Slight to severe erythema were noted in all animals at the 24-hour and 48-hour readings. Dryness of the skin was observed in almost all animals, and crusts were noted in some animals at the 72-hour reading. A slight oedema was also noted on both flanks in 12/20, 16/20 and 7/20 animals at the 24-, 48- and 72-hour readings, respectively.

These reactions were attributed to a sensitizing effect in 100% treated animals, whether the challenge application was performed with the test substance undiluted or at a concentration of 50% (w/w).

The guinea-pigs which were used in a recent study, showed a satisfactory sensitization response in 75% animals using a positive sensitizer (appendix 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 LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) (batch No. 802-9409-01) were observed in 100% guinea-pigs.

These reactions are not attributable to the solvent Isododecan, which failed to induce any sensitizing cutaneous reaction.

RESUME

A la demande de Elf Atochem Deutschland, Günzburg, Germany, le potentiel du produit LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) à induire une hypersensibilisation cutanée retardée après injection intradermique et application cutanée est évaluée chez le Cobaye selon la méthode de maximisation de Magnusson et Kligman conformément aux lignes directrices de l'O.C.D.E. (No. 406, 17th July 1992) et de la C.E.E. (92/69/C.E.E.. B₆). Le potentiel sensibilisant du solvant du produit testé, l'isododécane, est évalué en parallèle. L'étude est réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

Méthodes

Quarante cobayes sont répartis en 3 groupes : deux groupes témoins 1 et 2 (5 mâles et 5 femelles chacun) et un groupe traité 3 (10 mâles et 10 femelles).

Au jour 1, des injections intradermiques d'adjuvant de Freund mélangé avec le produit (groupe traité), l'huile de paraffine (groupe témoin 1) ou l'isododécane (groupe témoin 2) sont effectuées dans la région scapulaire.

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

Au jour 8, ce même site est traité par application topique de produit (groupe traité), d'huile de paraffine (groupe témoin 1) ou d'isododécane (groupe témoin 2) et recouvert d'un pansement occlusif pendant 48 heures.

Après une période de repos de 12 jours, les animaux du groupe témoin 1 reçoivent une application topique déclenchante de produit sur le flanc droit et d'isododecan sur le flanc gauche ; les animaux du groupe témoin 2 reçoivent une application déclenchante d'isododecan sur les flancs droit et gauche ; les animaux du groupe traité 3 reçoivent une application déclenchante de produit sur les flancs droit et gauche.

Les produits sont maintenus sous pansement occlusif pendant 24 heures. L'évaluation des réactions cutanées est effectuée environ 24, 48 et 72 heures plus tard.

Les concentrations de produits sont les suivantes :

Groupe témoin 1

Induction

- . application intradermique : huile de paraffine
- . application topique : huile de paraffine.

Application déclenchante

- . application topique :
LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) non dilué (flanc gauche)
isododécane non dilué (flanc droit).

Groupe témoin 2

Induction

- . application intradermique : isododécane à 50 % (p/p) dans l'huile de paraffine
- . application topique : isododécane non dilué.

Application déclenchante

- . application topique : isododécane à 50 % (p/p) dans l'huile de paraffine (flanc gauche)
isododécane non dilué (flanc droit).

Groupe traité 3

Induction

- . injections intradermiques :
LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) à 2,5 % (p/p)
dans un mélange d'isododécane et d'huile de paraffine.
- . application topique :
LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) non dilué.

Application déclenchante

- . application topique :
LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) à 50 % (p/p)
dans l'isododécane (flanc gauche)
LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) non dilué (flanc
droit).

A la fin de l'étude, les animaux sont sacrifiés et des prélèvements cutanés sont effectués au niveau des sites d'application déclenchante chez tous les animaux. Aucun examen histologique n'est réalisé sur les prélèvements cutanés.

La sensibilité de l'espèce Cobaye dans les conditions expérimentales du C.I.T. est contrôlée dans un essai récent réalisé avec un produit positif: le chloro-1 dinitro-2,4 benzène. Pendant la phase d'induction, le produit a été appliqué aux concentrations de 0,1 % (jour 1) et 1 % (jour 8). A l'application cutanée déclenchante, la concentration testée était de 1 % (p/p) sur le flanc droit.

Résultats

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

Dans les groupes témoins 1 et 2, aucune réaction cutanée n'est observée 24, 48 et 72 heures après l'enlèvement du pansement de l'application déclenchante cutanée. Seule une sécheresse de la peau est notée chez un animal du groupe 1 à chaque lecture, et chez presque tous les animaux des deux groupes au temps de lecture 72 heures.

Dans le groupe traité, d'importantes réactions cutanées, similaires sur les deux flancs, sont observées.

Des érythèmes légers à graves sont notés chez tous les animaux aux temps de lecture 24 et 48 heures. Une sécheresse de la peau est observée chez presque tous les animaux et des croûtes sont notées sur quelques animaux au temps de lecture 72 heures. Un léger oedème est également noté sur les deux flancs chez 12/20, 16/20 et 7/20 animaux, aux temps de lecture 24, 48 et 72 heures, respectivement.

Ces réactions sont attribuées à un effet sensibilisant chez 100 % des animaux traités, que l'application déclenchante ait été réalisée avec le produit non dilué ou à la concentration de 50 % (p/p).

Le témoin positif utilisé induit des réactions positives d'hypersensibilité cutanée chez 75 % des Cobayes (annexe 4).

Conclusion

Dans nos conditions expérimentales et selon la méthode de maximisation de Magnusson et Kligman, des réactions cutanées attribuables à un pouvoir sensibilisant du produit LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL) PEROXYDICARBONATE) sont observées chez 100 % des Cobayes.

Ces réactions ne sont pas attribuables au solvant isododécane, qui n'a entraîné l'apparition d'aucune réaction de sensibilisation.

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 LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) to induce delayed contact hypersensitivity in guinea-pigs, and to determine if possible reactions could be attributable to the solvent of this test substance.

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., B₆, 31st July 1992.

2. MATERIALS AND METHODS

2.1. TEST AND CONTROL SUBSTANCES

2.1.1 Test substance

The test substance LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) used in the study was supplied by Elf Atochem.

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

- . name:
 - protocol: LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE)
 - labelling: LUPEROX 223-M-75
- . batch number:
 - protocol and labelling: 802-9409-01
- . Cal. number:
 - protocol and labelling: 2418/95 and 2419/95
- . description: colourless liquid
- . container and quantity: two plastic flasks, each containing 0.1 kg, received at room temperature
- . date of receipt: 18 May 1995
- . storage conditions: at -20°C and protected from light
- . purity: 75.8%.

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.

(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).

2.1.2 Solvent

The solvent of the test substance, Isododecan, used in the study was supplied by Elf Atochem.

Documentation supplied by the Sponsor identified this product as follows:

- . name:
 - protocol and labelling: ISODODECAN
- . batch number:
 - protocol: none
 - labelling: 04.03.96
- . description: colourless liquid
- . container: one aluminium flask
- . date of receipt: 29 March 1996
- . storage conditions: at room temperature and protected from light.

At the beginning of the study, the analytical certificate was not available.

2.1.3 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. 7353 (Coopérative Pharmaceutique Française, 77000 Melun, France) for Isododecan.

Preparations of the test substance were made in Isododecan.

2.1.4 Preparation

The test substance was prepared at appropriate concentrations in the vehicle or Freund's complete adjuvant. The purity of the test substance was not taken into account for preparation of the different concentrations.

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

2.1.5 Other substances

The other substances used were sterile isotonic saline solution (0.9% NaCl), batch No. LD 4040 (Laboratoire Fresenius, 92316 Sèvres, France); Freund's complete adjuvant, batch No. 084H8800 (Sigma, 38297 Saint-Quentin-Fallavier, France); sodium laurylsulphate, batch No. 81H0841 (Sigma, 38297 Saint-Quentin-Fallavier, France) and vaseline, batch No. 0030 (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: 40 animals (20 males and 20 nulliparous and non-pregnant females).

Allocation of the animals to the groups: on day -1, the animals were weighed and randomly allocated to three groups: a control group 1 consisting of ten animals (five males and five females), a control group 2 consisting of ten animals (five males and five females) and a treated group 3 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 \pm standard deviation of 327 ± 18 g for the males and 347 ± 16 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: approximately 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 and ventilation) were checked monthly.

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 diet 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 for each product.

By intradermal route:

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

By cutaneous route:

- . 24 hours before treatment, both flank regions of the animals were clipped,
- . if necessary, the test substance or isododecan was prepared in an appropriate vehicle,
- . the test substance or isododecan (0.5 ml for each concentration) was applied to a dry gauze pad of approximately 4 cm² 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).

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 1	Control group 2
Anterior	1: FCA diluted at 50% (v/v) with 0.9% NaCl	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 2.5% (w/w) in Isododecan 50% (w/w) and paraffin oil 47.5% (w/w)	2: paraffin oil	2: Isododecan at 50% (w/w) in paraffin oil
Posterior	3: mixture of 50/50 (w/v) of 1 and 2	3: mixture of 50/50 (w/v) of 1 and 2	3: 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 or solvent 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 1

. application of 0.5 ml of the vehicle.

Control group 2

. application of 0.5 ml of Isododecan at the chosen concentration.

Treated group 3

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

The test substance or Isododecan and the vehicle were prepared on a dry gauze pad (Coopérative Pharmaceutique Française, 77000 Melun, 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 of the control group 1 received an application of 0.5 ml of the test substance at the chosen concentration to the posterior left flank, and 0.5 ml of Isododecan to the posterior right flank. The animals of the control group 2 received an application of 0.5 ml of isododecan at the chosen concentrations to the posterior right and left flanks.

The treated animals received an application of 0.5 ml of the test substance at the chosen concentrations to the posterior right and left flanks.

These applications were performed using a 1 ml glass syringe (0.01 ml graduations, Record: Carrieri, 75005 Paris, France). The test substance, Isododecan and vehicle were prepared on a dry gauze pad (Coopérative Pharmaceutique Française, 77000 Melun, France), then applied to a 4 cm² (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).

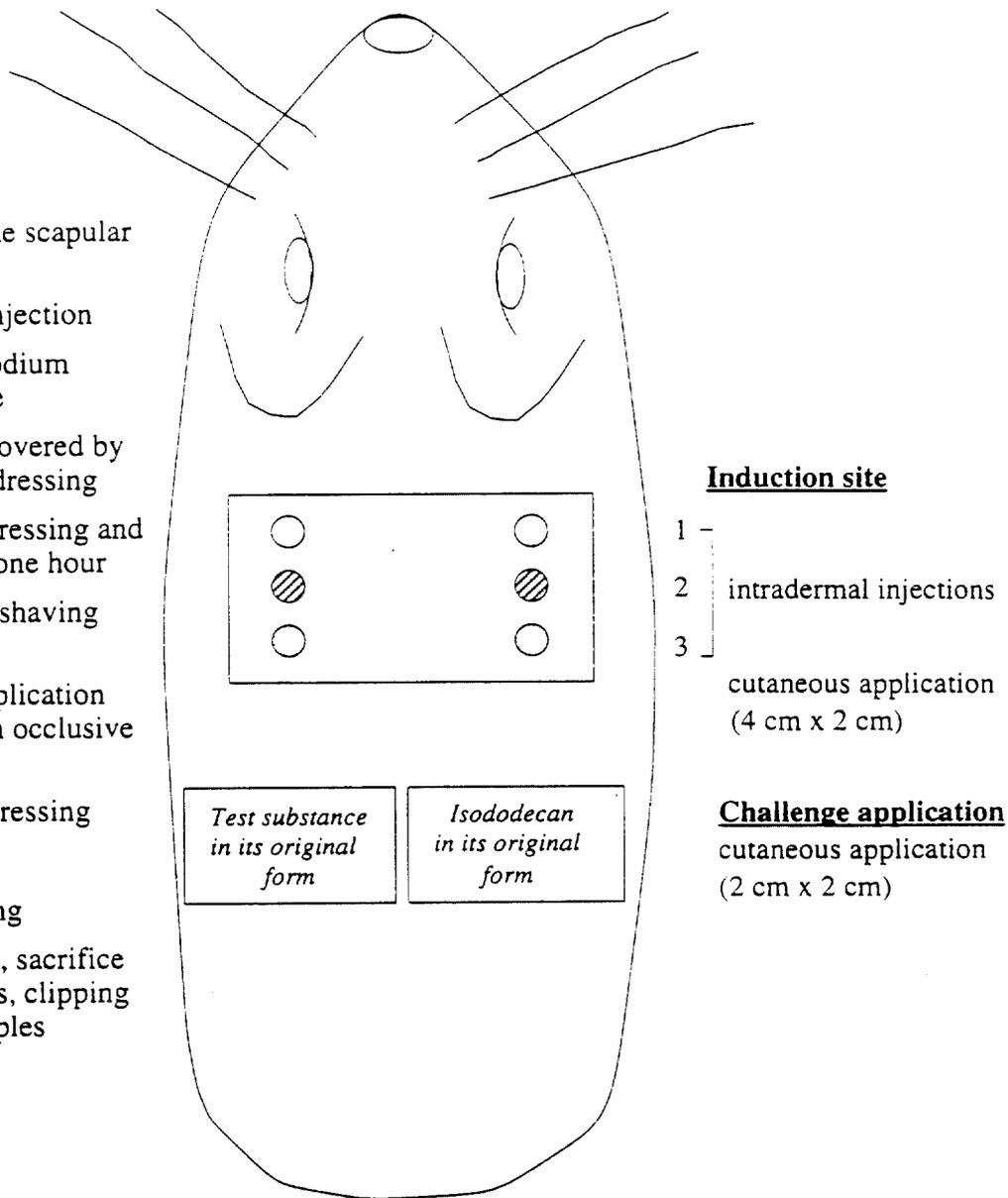
On removal of the dressing, if present, any residual test substance was removed by means of a dry or a moistened gauze pad.

2.4. SUMMARY DIAGRAMS

Figure 1: control group 1

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
- Day 26 Third scoring, sacrifice of the animals, clipping and skin samples

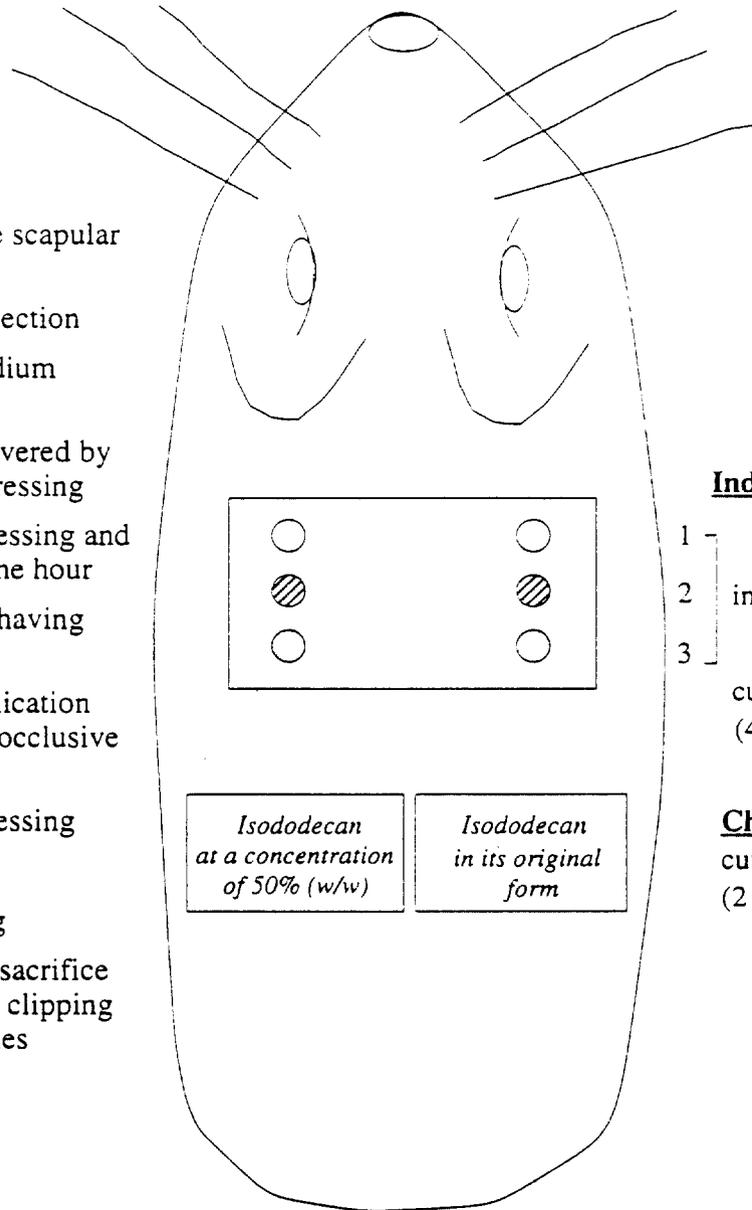


- Intradermal injections
- 1 } 50% Freund's complete adjuvant and sterile isotonic solution (0.9% NaCl)
 - ◐ 2 } vehicle
 - 3 } 1 + 2, 50/50 (w/v)

Figure 2: control group 2

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
- Day 26 Third 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

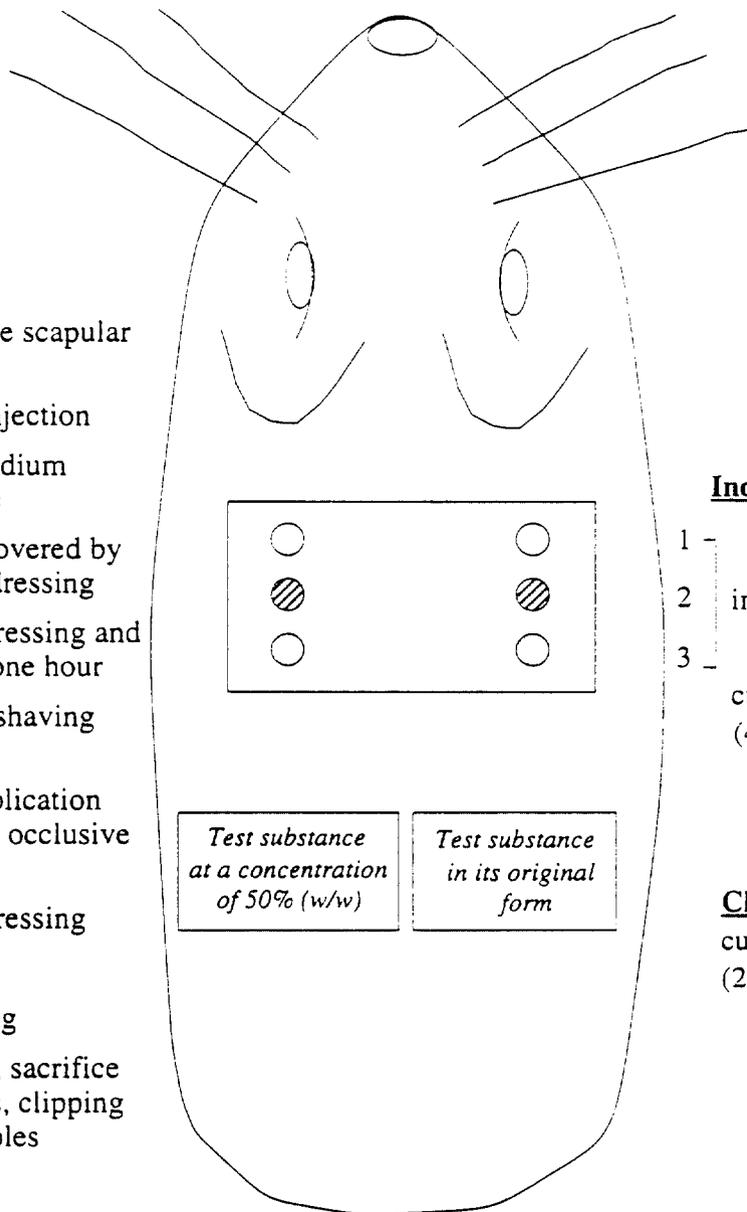
- cutaneous application (2 cm x 2 cm)

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

Figure 3: treated group 3

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
- Day 26 Third scoring, sacrifice of the animals, clipping and skin samples



Induction site

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

Challenge applications

- cutaneous application (2 cm x 2 cm)

- 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)
- Intradermal injections

2.5. SCORING OF CUTANEOUS REACTIONS

Twenty-four, 48 and 72 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 CO₂ 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 and/or oedema ≥ 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

(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	25 April 1996	-8
Weighing and allocation of the animals into groups	2 May 1996	-1
Weighing, induction by intradermal injection	3 May 1996	1
Laurylsulfate application	9 May 1996	7
Weighing, induction by cutaneous route	10 May 1996	8
Removal of occlusive dressings and scoring of local reactions after one hour	12 May 1996	10
Weighing	17 May 1996	15
Challenge cutaneous application	24 May 1996	22
Removal of occlusive dressings	25 May 1996	23
Scoring of cutaneous reactions after		
. 24 hours	26 May 1996	24
. 48 hours	27 May 1996	25
. 72 hours	28 May 1996	26
Weighing, sacrifice of the animals and skin samples	28 May 1996	26

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

Several tests were performed in order to determine the concentration to be used in the main study.

	Animal number	Concentration of the test substance % (w/w)	Scoring after treatment		
			24 hours	48 hours	6 days
LUPEROX in Isododecan	male 01	0.1	slight irritation	slight irritation	tissular destruction
		1	irritation	slight irritation	tissular destruction
		2.5	irritation	slight irritation	tissular destruction
	female 01	0.1	slight irritation	slight irritation	tissular destruction
		1	irritation	slight irritation	tissular destruction
		2.5	irritation	slight irritation	tissular destruction
Isododecan in paraffin oil	male 02	10	slight irritation	slight irritation	slight irritation
		25	slight irritation	slight irritation	slight irritation
		50	irritation	irritation	slight irritation
	female 02	10	slight irritation	slight irritation	slight irritation
		25	slight irritation	slight irritation	slight irritation
		50	irritation	irritation	slight irritation

Concentration chosen for the main study was 50% (w/w) for Isododecan and 2.5% (w/w) for LUPEROX.

3.1.2 Application by cutaneous route

Several tests were performed in order to determine the concentrations to be used in the main study.

Animal number	Concentration of the test substance %		Scoring after removal of the dressing (1)			
			24 hours		48 hours	
			E	O	E	O
<u>LUPEROX</u>						
male 01	100	RF	0	0	0	0
	100	LF	0	0	0	0
female 01	100	RF	0	0	0	0
	100	LF	0	0	0	0
<u>Isododecan</u>						
male 02	100	RF	0	0	0	0
	100	LF	0	0	0	0
female 02	100	RF	0	0	0	0
	100	LF	0	0	0	0

E : erythema

RF: right flank

O : oedema

LF: left flank

(1) : No residual test substance was observed.

Isododecan

Concentration chosen for the topical application of the induction phase (day 8) was 100%. For the challenge application, it was 50% (w/w) and 100%.

LUPEROX

Concentration chosen for the topical application of the induction phase (day 8) was 100%. For the challenge application, it was 50% (w/w) and 100%.

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 4 and 5, 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 groups 1 and 2, and crusts were noted in almost all animals of group 3.

3.2.2.2 Challenge application

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

Skin reactions were as follows:

Sex	Animal number	Control group 1											
		24 hours				48 hours				72 hours			
		Erythema		Oedema		Erythema		Oedema		Erythema		Oedema	
LF	RF	LF	RF	LF	RF	LF	RF	LF	RF	LF	RF		
Male	61	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	62	0/S	0	0	0	0/S	0	0	0	0/S	0/S	0	0
	63	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	64	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	65	0	0	0	0	0	0	0	0	0/S	0/S	0	0
Female	81	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	82	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	83	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	84	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	85	0	0	0	0	0	0	0	0	LS	0	0	0

LF: left flank
 RF: right flank
 S : dryness of the skin
 LS: scoring masked by dryness of the skin

Control group 2													
Sex	Animal number	24 hours				48 hours				72 hours			
		Erythema		Oedema		Erythema		Oedema		Erythema		Oedema	
		LF	RF	LF	RF	LF	RF	LF	RF	LF	RF	LF	RF
Male	66	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	67	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	68	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	69	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	70	0	0	0	0	0	0	0	0	0/S	0	0	0
Female	86	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	87	0	0	0	0	0	0	0	0	0	0/S	0	0
	88	0	0	0	0	0	0	0	0	0	0/S	0	0
	89	0	0	0	0	0	0	0	0	0/S	0/S	0	0
	90	0	0	0	0	0	0	0	0	0/S	0/S	0	0

LF : left flank
 RF : right flank
 S : dryness of the skin

Treated group 3													
Sex	Animal number	24 hours				48 hours				72 hours			
		Erythema		Oedema		Erythema		Oedema		Erythema		Oedema	
		LF	RF	LF	RF	LF	RF	LF	RF	LF	RF	LF	RF
Male	71	2/S/A	2/S/A	0	0	LS	LS	2	2	0/S	0/S	0	2
	72	2/S/A	2/S/A	0	0	LS	LS	2	2	0/S	0/S	2	2
	73	2	2	2	2	2	2	2	2	LS/A	0/S	2	2
	74	2	2	0	0	2/S	2/S	0	0	0/S	0/S/A	0	0
	75	1	1	0	0	2/S	1/S	0	0	LS/A	LS	0	0
	76	2	2	0	0	1/S	2/S	0	0	LS/A	0/S	0	0
	77	3/S/A	3/S/A	2	2	4/S	4/S	2	2	LS/A	2/S	2	2
	78	1	2	0	2	2/S	3/S	2	2	LS/A	LS/A	2	2
	79	2	2	0	0	3/S	3/S	2	2	0/S	0/S	0	0
	80	1	2	0	0	LS	2/S	2	2	0/S	0/S/A	0	0
Female	91	2/S	2/S	2	2	LS	LS	2	2	LS	LS	0	0
	92	2/S	2/S	2	2	3/S	4/S	2	2	LS/A	LS/A	0	0
	93	1	2	2	2	2/S	2/S	2	2	LS/A	LS	0	0
	94	2	2	2	2	3/S	3/S	2	2	LS/A	LS/A	0	0
	95	2	2	2	2	LS	LS	2	2	LS	LS	2	2
	96	3/S	3/S	2	2	3/S	LS	2	2	LS/A	LS	0	0
	97	2	2	2	2	LS	LS	2	2	0/S	LS	0	0
	98	2	2	2	2	LS	LS	2	2	LS	LS	0	0
	99	3	3	0	0	2/S	2/S	0	0	LS/A	0/S	0	0
	100	2/S	3	2	2	LS	LS	2	2	LS	LS	2	2

LF : left flank
 RF : right flank
 S : dryness of the skin
 A : crusts
 LS : scoring masked by a severe dryness of the skin

In the control groups 1 and 2, no cutaneous reactions were observed 24, 48 and 72 hours after removal of the dressings of the challenge cutaneous application. Only dryness of the skin was noted in one animal from group 1 at each reading, and in almost all animals from both groups at the 72-hour reading.

In the treated group, marked cutaneous reactions, similar on both flanks, were observed. Slight to severe erythema (grade 1 to 4) were noted in all animals at the 24-hours and 48-hours readings. Dryness of the skin was observed in almost all animals, and crusts were noted in some animals at the 72-hour reading. A slight oedema (grade 2) was also noted on both flanks in 12/20, 16/20 and 7/20 animals at the 24-, 48- and 72-hour readings, respectively.

These reactions were attributed to a sensitizing effect in 100% treated animals, whether the challenge application was performed with the test substance undiluted or at a concentration of 50% (w/w).

3.2.3 Pathology: 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 LUPEROX 223-M-75 (DI-(2-ETHYLHEXYL)-PEROXYDICARBONATE) (batch No. 802-9409-01) were observed in 100% guinea-pigs.

These reactions are not attributable to the solvent Isododecan, which failed to induce any sensitizing cutaneous reaction.

Figure 4: Male body weight (g)

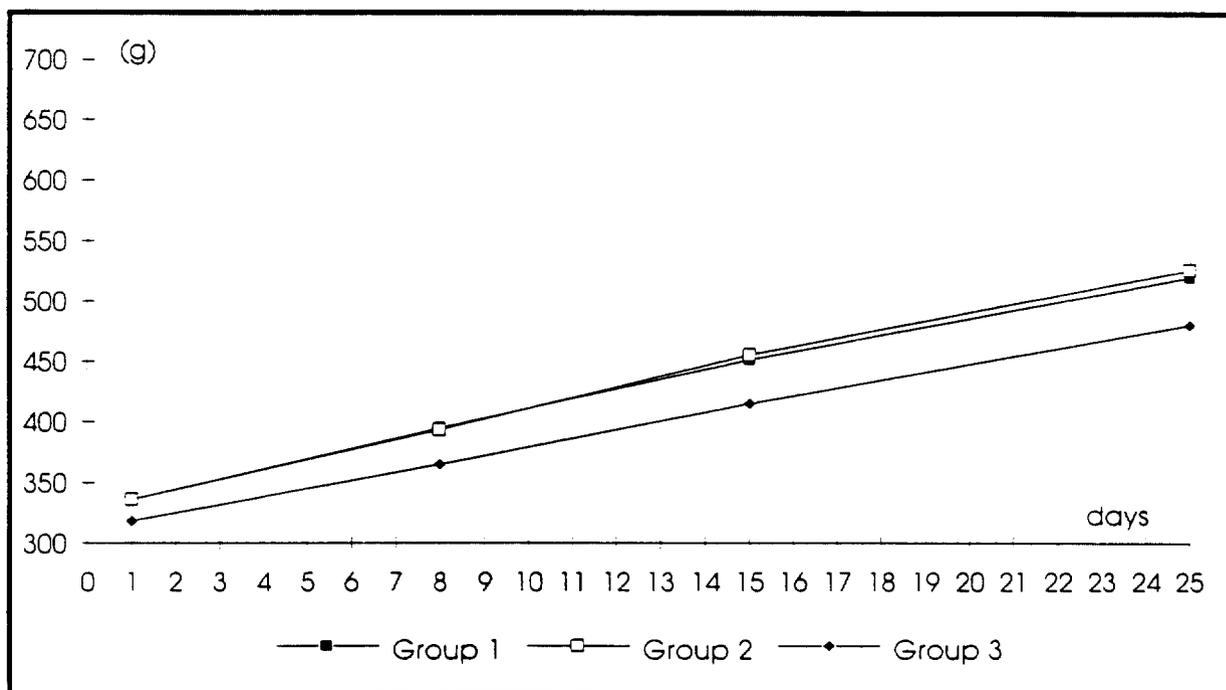
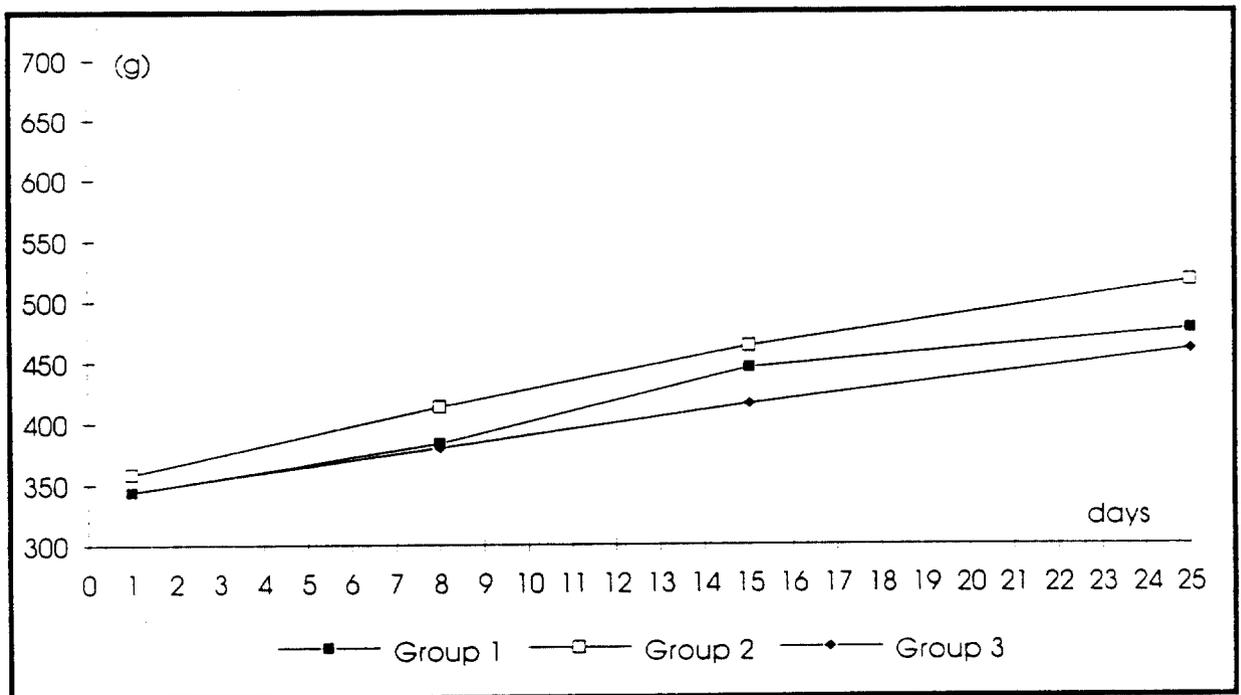


Figure 5: Female body weight (g)



APPENDICES

1. Test article description and analytical certificate of the test substance

TOXICOLOGY DEPARTMENT
CONFIDENTIAL
23 May 1995

elf atochem s.a.

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

TEST ARTICLE DESCRIPTION

LUPEROX 223-M-75
(Di-(2-ethylhexyl)-peroxydicarbonate)

IDENTITY

Test article name : LUPEROX 223-M-75
Chemical name : Peroxydicarbonic acid, bis(2-ethylhexyl)ester
CAS number : 16111-62-9
EINECS number : 2402824
Molecular formula : C₁₈H₃₄O₆
Molecular weight : 346.52
Purity : 75.8% in hydrocarbon
Origin and batch : Elf Atochem Deutschland, Günzburg, 802-9409-01
Elf Atochem filing number : CAL 2418/95 & 2419/95

PHYSICAL AND CHEMICAL PROPERTIES

Appearance : Liquid
Specific gravity : 0.91
Flash point : > 55°C (open cup)
Solubility : not soluble in water

TOXICOLOGICAL INFORMATIONS AND USE SAFETY

See Safety data Sheet.

STORAGE AND DISPOSAL

Storage : in dark and < -15°C
Disposal date : After May 1996
Disposal : See Safety data Sheet

Elf Atochem Deutschland GmbH
 Niederlassung LUPEROX Gürnberg
 Demzinger Straße 7 · D-89312 Gürnberg
 Postfach 13 54 · D-89303 Gürnberg
 Tel. (0 82 21) 98-0 · Telefax (0 82 21) 98-166 · Telex 531 121



250001WQ

Elf Atochem Deutschland GmbH · Postfach 13 54 · D-89303 Gürnberg
 Elf Atochem S.A.
 Centre d'Application de Levallois
 Attn: Mme Varlet
 05, rue Danton
 F - 92300 Levallois Perret

Gürnberg, 19.05.95
 Rechnung Nr. vom 19.05.95
 Invoice No. of
 Facture No. du
 Fattura No. del

Ihre Bestellung: Ihre Bestellung:
 Your Order: Your Order:
 Votre Commande: Votre Commande:
 Vs. Ordine: Vs. Ordine: fax du 10.01.95

Lieferadresse: Elf Atochem S.A.
 Delivery Address: Centre d'Application
 Adresse de livraison: Centre d'Application
 Destinazione:

ANALYSENZERTIFIKAT
 CERTIFICATE OF ANALYSIS
 CERTIFICAT D'ANALYSE
 CERTIFICATO D'ANALISI

2x100g \approx LUPEROX 223 M 75
 di(2-ethylhexyl)-peroxycarbonate
 en hydrocarbure

Chargen Nr. Batch No. Charge No. Lotto No.	Gebindezahl No. of Units Nombre de Unités No. di fusti	Peroxidgehalt Assay teneur en Peroxide Contenuto di Peroxido			Cl		
802-9409-01	2	75,8			§	0,006	

Elf Atochem Deutschland GmbH
 Niederlassung LUPEROX Gürnberg

[Handwritten signatures]

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.

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	331	327	69	396	31	427	64	491
		62	335	355	45	400	46	446	63	509
		63	330	339	77	416	82	498	55	553
		64	320	335	44	379	73	452	79	531
		65	310	322	61	383	53	436	83	519
		M	325	336	59	395	57	452	69	521
		SD	10	13	15	15	21	28	12	23
2	Male	66	325	332	64	396	58	454	83	537
		67	318	348	60	408	55	463	76	539
		68	333	347	69	416	70	486	85	571
		69	356	355	75	430	54	484	50	534
		70	297	298	18	316	77	393	62	455
		M	326	336	57	393	63	456	71	527
		SD	22	23	23	45	10	38	15	43
3	Male	71	312	329	50	379	22	401	64	465
		72	319	325	41	366	55	421	67	488
		73	290	290	64	354	50	404	52	456
		74	310	314	11	325	51	376	70	446
		75	300	305	35	340	64	404	72	476
		76	300	317	59	376	58	434	61	495
		77	317	323	54	377	61	438	91	529
		78	302	314	42	356	41	397	56	453
		79	315	326	54	380	32	412	71	483
		80	325	337	56	393	73	466	57	523
		M	309	318	47	365	51	415	66	481
		SD	11	13	15	21	15	25	11	28

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

INDIVIDUAL BODY WEIGHT VALUES
(g)

Groups	Sex	Animals	Days							
			-1	1	(1)	8	(1)	15	(1)	25
1	Female	81	321	340	55	395	61	456	44	500
		82	303	322	34	356	67	423	64	487
		83	340	348	30	378	59	437	-77	360
		84	346	362	56	418	53	471	86	557
		85	330	345	26	371	70	441	39	480
		M	328	343	40	384	62	446	31	477
		SD	17	14	14	24	7	18	63	72
2	Female	86	352	363	57	420	31	451	43	494
		87	342	357	63	420	64	484	66	550
		88	344	343	70	413	48	461	58	519
		89	346	344	56	400	69	469	74	543
		90	372	385	30	415	35	450	27	477
		M	351	358	55	414	49	463	54	517
		SD	12	17	15	8	17	14	19	31
3	Female	91	336	346	56	402	54	456	64	520
		92	352	354	58	412	41	453	7	460
		93	314	326	50	376	48	424	33	457
		94	318	319	-9	310	48	358	68	426
		95	316	341	21	362	44	406	63	469
		96	331	332	40	372	46	418	15	433
		97	318	340	30	370	-4	366	68	434
		98	346	359	49	408	12	420	5	425
		99	348	364	33	397	54	451	50	501
		100	341	359	31	390	16	406	74	480
		M	332	344	36	380	36	416	45	461
SD	15	15	20	30	20	34	27	33		

(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'Elevage Lebeau) to a positive control test article

Method : Magnusson and Kligman
 Test substance : 2,4-dinitro chlorobenzene
 C.I.T. Study - Date : (CIT/Study No. 13633 TSG) - December 1995
 Number of animals : ten males and ten females
 Induction : 0.1% intradermal route day 1
 1% 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 chlorobenzene at a concentration of 1% (w/w) induced positive skin sensitization reactions in 75% 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	Male	81	0	1/C	0	0	0	0/C/S	0	0	-	-
		82	0	2/S	0	0	0	1/C/S	0	0	-	+
		83	0	1	0	0	0	0/C/S	0	0	-	-
		84	0	2	0	2	0	0/C/S	0	0	-	+
		85	0	2/C/S	0	0	0	2/C/A	0	0	-	+
		86	0	1	0	0	0	1/C/S	0	0	-	+
		87	0	2/A	0	2	0	4/A/C	0	0	-	+
		88	0	2	0	0	0	0/C/S	0	0	-	+
		89	0	1	0	0	0	0/S	0	0	-	-
	90	0	2	0	4	0	2/C/S	0	0	-	+	
	Female	96	0	2	0	2	0	2/C/S	0	0	-	+
		97	0	1	0	0	0	0/S	0	0	-	-
		98	0	1/C	0	0	0	0/C/S	0	0	-	-
		99	0	2	0	0	0	LS	0	0	-	+
		100	0	2	0	2	0	0/C/S	0	0	-	+
		101	0	2/A	0	0	0	LS/A	0	0	-	+
		102	0	2/S	0	0	0	2/C/S	0	0	-	+
		103	0	2	0	0	0	2/C/S	0	0	-	+
104		0	3	0	2	0	4/S	0	2	-	+	
105	0	1	0	0	0	LS	0	0	-	+		

- : negative
- + : hypersensitizing reactions
- C : yellow colouration of the skin due to the test substance
- S : dryness of the skin
- A : crusts
- LS: scoring masked by a marked dryness of the skin
- LF: left flank
- RF: right flank