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D - 6700 Ludwigshafen  
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TEST ARTICLE : UVINUL T 150 (Batch 19-0518)

BASF PROJECT : N° 99H0755/909013

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TEST TO EVALUATE THE PHOTOTOXIC AND PHOTOALLERGIC  
POTENTIALS BY TOPICAL APPLICATIONS IN THE GUINEA-PIG

32 page-document

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GENERAL INFORMATION

- TEST ARTICLE : UVINUL T 150 (Batch 19-0518)
  
- STUDY PERFORMED : TEST TO EVALUATE THE PHOTOTOXIC AND PHOTOALLERGIC POTENTIALS BY TOPICAL APPLICATIONS IN THE GUINEA-PIG (P.P.T.)
  
- STUDY SPONSOR
  - . Address : BASF  
D - 6700 Ludwigshafen  
WEST-GERMANY
  
  - . Study monitor : Dr. SCHILLING
  
- TESTING FACILITY
  - . Address : HAZLETON FRANCE  
Les Oncins - B.P. 0118  
69593 L'ARBRESLE CEDEX, FRANCE.
  
  - . Study director : O. MERCIER  
Docteur-Ingénieur en Neurosciences, D.E.S.S. de  
Pharmacologie Expérimentale, Pharmacocinétique et  
Toxicologie Expérimentale.
  
- PROTOCOL N° 2791D of 22 January 1991, accepted on 1st February 1991
  
- STUDY TIMETABLE
  - . Start of the study (signing of protocol by the Study Director) :  
22 January 1991
  - . End of the study (signing of final report by the Study Director) :  
18 June 1991



QUALITY ASSURANCE

This study was performed in accordance with the Good Laboratory Practice - GLP - Principles described in the Decision of the O.E.C.D. Council concerning the Mutual Acceptance of Data in the Assessment of chemicals - C/81/30 (final) Annex II dated 12 May 1981.

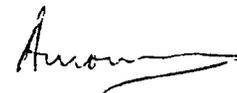
It was inspected by the Quality Assurance Department of Hazleton-France on the dates indicated below.

The findings of these audits were submitted as reports to the General Management, to the Study Director and to those involved in performing the experiment.

The data presented in this report are an accurate reflection of the raw data.

Audits	Dates of audits	Dates of reports to Management
Test system : - housing, administration of test article	26 February 1991	14 March 1991
Draft report vs Raw data	6 June 1991	7 June 1991
Final report	11 June 1991	18 June 1991

Signature :

Name : Leif MODEWEG  
Title : General Manager

Anne-Marie AUROUSSEAU  
Quality Assurance  
Auditor of the report

Date :

26 June 1991

27 June 1991

HAZLETON FRANCE  
Les Oncins - BP 0118 - 69593 L'Arbresle Cedex - France

STUDY SPONSOR : BASF

TEST ARTICLE : UVINUL T 150  
(Batch 19-0518)

S U M M A R Y

§ - TEST TO EVALUATE THE PHOTOTOXIC AND PHOTOALLERGIC POTENTIALS BY TOPICAL APPLICATIONS IN THE GUINEA-PIG

(According to the protocol published by Guillot-Martini-Gonnet-Loquerie-Cotte)

PROTOCOL

Evaluation of the cutaneous phototoxic and photoallergic potential of the test article was performed in the albino Dunkin-Hartley guinea-pig according to a maximized protocol using 25 animals of both sexes, allocated in one control group of 5 treated but non-irradiated guinea-pigs and one group of 20 treated and irradiated animals.

All the applications were carried out with the test article as supplied and at the dose level of 0.33 g (quantity corresponding to a volume of 0.50 ml).

In the treated and irradiated group, each application of the test article was followed by irradiation with U.V.B. and/or U.V.A. lamps.

Cutaneous macroscopic examinations were carried out according to the Draize scale, 6 and 24 hours after the 1st irradiation (evaluation of phototoxicity), then 6, 24 and 48 hours after the challenge irradiation (evaluation of photoallergy).

RESULTS AND CONCLUSION

The examinations did not show evidence of any pathological lesion of phototoxic or photoallergic type in the 20 treated and irradiated guinea-pigs. No cutaneous abnormality was noted in the 5 control guinea-pigs.

From the results obtained under the experimental conditions employed, the test article did not provoke any phototoxicity nor any photoallergy reaction.

*P/*  
*BTIFFROY*  
*Scientific*  
*Manager*  
O. MERCIER  
Study Director

18 June 1991

INFORMATION RELATIVE TO SUBSTANCESTEST ARTICLE

- . Identification : UVINUL T 150
- . Identification for the study : 00625 A1 001
- . Presentation : white heterogeneous powder
- . Batch Number : 19-0518
- . Packaging : plastic container
- . Quantity and date received : about 500 g on 24 January 1991
- . Storage : room temperature
- . Volumic mass = 0.6625 g/ml, considered as 0.66 g/ml for the study  
Conditions of measurement : weighing of 1 ml of the test article, using a Mettler AE 200 (balance (d = 0.1 mg).  
T = 20°C
- . pH = 6.4  
Conditions of measurement : the measurement was carried out under magnetic stirring. The test article was prepared in a 17 % (W/W) suspension in purified water (house preparation of 11 March 1991) - pH = 5.7 at 23.4°C.  
pH-meter Bioblock 93317 (p = 0.02 pH)  
Electrode Ingold (Ref. 405-DXK-S8)  
T = 23.5°

SUBSTANCES ADMINISTERED

- . Freund's complete adjuvant : batch 784560, peremption August 1993 (Difco Laboratories - Detroit - Michigan - USA) in a 50 % (V/V) dilution in an isotonic injectable solution : 0.9 % NaCl - batch 9314A2, peremption March 1995 (Laboratoire Aguettant - Lyon - France)
- . Test article as supplied.

N.B. : the stability of the test article will be analysed by the Sponsor.

TEST TO EVALUATE THE PHOTOTOXIC AND PHOTOALLERGIC  
POTENTIALS BY TOPICAL APPLICATIONS IN THE GUINEA-PIG

(P.P.T.)

(According to the protocol published by

GUILLOT - MARTINI - GONNET - LOQUERIE - COTTE)

EXPERIMENTAL PROTOCOL

1. STUDY OBJECTIVE

This method is used to evaluate, according to a maximised protocol, the cutaneous phototoxic and photoallergic potentials of a test article in the albino guinea-pig. The applications are performed under an occlusive patch on skin which is then subjected to ultra-violet radiations. Induction of photoallergy involved the use of Freund's complete adjuvant by the intradermal route.

2. PRINCIPLE

2.1. Phototoxicity

- a single application under an occlusive patch for 1 hour 30 minutes of test article to the clipped and depilated back (anterior area) of 20 guinea-pigs, at the maximum dose level or concentration recognized as non-irritant during a preliminary study.

- Irradiation of the treated skin, immediately after removal of the occlusive patch.

- The phototoxic reaction is evaluated by macroscopic examinations and in certain cases by histopathological examinations of the cutaneous lesions observed 24 hours after irradiation. Comparison is made on the same animal with an area of skin which is irradiated and not treated and slightly erythematous at 6 hours (irradiation corresponding to the Minimal Erythematous Dose level : M.E.D.) and with a control group of 5 guinea-pigs which are treated but not irradiated.

2.2. Photoallergy

- Induction period, this involves "preparatory" or "photosensitizing" contacts between the organism and photoallergen which provokes the photoallergic process without provoking clinical manifestations of hypersensitivity :

. 4 applications (the 1st one being specific of phototoxicity) under an occlusive patch for 1 hour 30 minutes of a determined quantity of test article to the clipped and depilated back (anterior area) of guinea-pigs ;

- . 4 simultaneous intradermal injections of Freund's complete adjuvant into both sides of the application site (before the second application) ;
- . 4 irradiations of the treated skin immediately after removal of the occlusive patch corresponding to each one of the 4 applications.

- Rest period, or incubation period during which possible cellular transformations continue which bring the modification of reactivity :

- . 14 days without treatment.

- "Challenge" phase, corresponding to contact between the organism and the photoallergen which provokes clinical manifestation of photoallergy :

- . Application of the test article to clipped and depilated skin which was not previously treated (posterior area of the back) at a dose level or concentration which does not provoke any pathological orthoergic cutaneous reaction, by the enclosed epicutaneous route for 1 hour 30 minutes.
- . Irradiation of the treated skin immediately after removal of the occlusive patch in order to evaluate the possible photoallergic potential of the test article.

The photoallergic reaction is determined by macroscopic examinations and in certain cases by histopathological examinations of the cutaneous lesions observed at the time of the "challenge" application, 6 and possibly 24 and 48 hours after irradiation. Comparison is made on the same animal with the 1st application (corresponding to the phototoxicity), and with the guinea-pigs of the treated but not irradiated control group.

### 3. TEST SYSTEM

- Species, strain : Dunkin-Hartley albino guinea-pig.
- Reason for choice of species : the guinea-pig is recognized as the animal species most sensitive for the evaluation of phototoxic and photoallergic potentials. This species is traditionally used in this type of study.
- Supplier : Elevage Lebeau (78950 Gambais - France)
- Age : young adult.
- Weight at the beginning of treatment :
  - . 407 g to 498 g for the preliminary study ;
  - . 305 g to 409 g for the main study (the weights are reported in appendix).
- Number and sex :
  - . preliminary study : 2 males, 2 non-pregnant females ;
  - . main study : 13 males, 12 non-pregnant females :
    - Group I : 3 males, 2 females, treated but not irradiated.
    - Group II : 10 males, 10 females ; two complementary guinea-pigs were treated and irradiated (1 male and 1 female) as replacements in the event of any non-treatment related deaths.

4. HOUSING AND ENVIRONMENT

- Cages : housing by sex in groups of 5 or 6 (or 2 or 3 for the preliminary studies and for group I of main study) in polystyrene cages with perforated flooring (internal dimensions 560 x 355 x 315 mm).
- Temperature : 19.5 to 23.5°C (see page 18).
- Humidity : 42 to 76 % R.H. (see page 18).
- Lighting : artificial 12 hours out of 24.

5. DIET AND DRINKING WATER

- Diet : complete pelleted guinea-pig maintenance diet ad libitum (U.A.R. formula 114 - U.A.R., Villemoisson - 91360 Epinay S/Orge - France). Bacteriological and chemical analysis performed by the supplier.
- Water : filtered and softened mains drinking water ad libitum (automatic dispenser). Bacteriological and chemical analysis performed twice a year.

6. PRE-EXPERIMENTAL PROCEDURES

- Acclimatisation period :
  - . preliminary study : 37 days before the start of treatment ;
  - . main study : 7 days before the start of treatment.
- Clinical examinations : on arrival, then before the start of treatment to ensure only healthy animals are retained for the study ; any guinea-pig presenting cutaneous lesions was eliminated from the study.
- Identification :
  - . animals : a metal tag engraved with a unique number placed through the ear pinna, before the start of treatment ;
  - . cages : colour-coded label with the number and sex of the guinea-pigs, the code number of the test article, the starting and finishing dates of the test (a single label per group).
- Selection and allocation of animals : they were taken from the animal house stock after the acclimatisation period and allocated as they came to hand, sex by sex and group by group, one by one, in order to have one animal per group at each time until the required number per group is achieved.
- Preparation of the treated area : the day before the 1st application of test article, the back and flanks of each animal were clipped carefully with electric clipper and then depilated with cream. At the time of application only those animals presenting healthy skin, free from any macroscopic irritation were retained for the study.

7. PRELIMINARY STUDY

This test for the evaluation of phototoxic and photoallergic potentials could only be conducted if the test article did not provoke a pathological orthoergic cutaneous reaction when it was administered as supplied or at least at its maximum concentration of use.

The objective of this preliminary study was thus to determine the maximum non-irritant dose level or concentration in the guinea-pig, after one application under an occlusive patch for 1 hour 30 minutes to the clipped and depilated skin but not exposed to U.V. radiations.

7.1. Experimental design7.1.1. Group and dose levels  
-----

- Group : one group of 2 males and 2 females.

- Dose levels : 0.33 g and 0.17 g (quantities corresponding to volumes of 0.50 and 0.25 ml) per animal of the test article as supplied.

- Reason for the choice of the dose level : 0.50 ml is the maximum dose level which can be applied under the occlusive patch used.

7.1.2. Route and methods of administration  
-----

- Route : cutaneous.

- Reason for the choice of route : possible route of exposure in man.

- Methods of administration : the 2 dose levels were applied to the previously clipped and depilated skin of the dorsal region of the same animals under an occlusive patch for 1 hour 30 minutes, according to the methods described in 8.1.1., but the gauze being moistened with sterile Codex liquid paraffin instead of water (see page 18).

- After removal of the patch, the surplus test article was wiped with a gauze moistened with purified water.

7.1.3. Frequence of administration  
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The test article was applied once only.

7.2. Observations and examinations performed7.2.1. Reading intervals  
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The cutaneous examinations were performed 6, 24 and 48 hours after removal of the patches.

## 7.2.2. Description and evaluation of the macroscopic cutaneous reactions observed

-----  
 These examinations were always performed under the same conditions particularly with reference to lighting.

The cutaneous lesions were evaluated for each of the previously mentioned intervals and for each guinea-pig according to the following scales :

## Erythematous lesions

. No erythema .....	0
. Slight erythema (hardly visible) .....	1
. Clearly visible erythema .....	2
. Marked erythema .....	3
. Severe erythema (crimson red) with or without eschar formation (deep lesions) and all lesions interpreted as serious cutaneous reactions such as burning, necrosis etc .....	4

## Oedematous lesions

. No oedema .....	0
. Very slight oedema (hardly visible) .....	1
. Slight oedema (well defined shape, obvious swelling) .....	2
. Marked oedema (approximately thickness 1 mm) .....	3
. Severe oedema (thickness greater than 1 mm and a surface area greater than the application area) .....	4

## 7.2.3. Histopathological examinations of the skin

-----  
 As no macroscopic reaction was observed in the 4 guinea-pigs examined at the dose level of 0.33 g, no cutaneous biopsy was taken for histopathological examination.

8. MAIN STUDY

Animal weighing : Day 1 (1st application) and Day 23.

° Groups :

Two groups treated with the test article were allocated as follows :

- GROUP I : animals treated but not irradiated : 3 males and 2 females.  
 These 5 animals received the test article and Freund's adjuvant under the same conditions as the guinea-pigs in GROUP II, but were not subjected to irradiation : this was to verify that any possible cutaneous reaction in the irradiated animals was a phototoxic or photoallergic reaction and not due to any other cause.

- GROUP II : animals treated and irradiated : 10 (+ 1) males and 10 (+ 1) females.  
 These 22 guinea-pigs received the test article and Freund's adjuvant followed by exposure to radiations from Ultra-Violet lamps.

° Route of administration :

- Route : cutaneous.

- Reason for the choice of route : possible route of exposure in man.

8.1. Evaluation of phototoxic potential

8.1.1. Experimental design  
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- Application area : retro-scapular region.

- Time of administration : Day 1 (Day-1 : clipping and depilation with cream.

- Dose level : 0.33 g (quantity corresponding to a volume of 0.5 ml) per animal of the test article as supplied (maximum non-irritant dose level determined at the time of the preliminary study).

- Methods of administration :

. The test article was applied directly to the animal skin over an area of approximately 4 cm<sup>2</sup>. It was then covered with a square of 8 layer Codex hydrophilic gauze about 2 cm x 2 cm, previously moistened with 0.5 ml of purified water.

. The test article and the gauze square were maintained in contact with the skin with the aid of an adhesive hypoallergenic "patch" (Hazleton France/Urgo), composed of an occlusive disc of 28 mm diameter attached to the skin by means of an adhesive and microporous border 10 mm wide.

. For the 1st (Phototoxicity) and the last applications (Photoallergy) this "patch" was covered with a sheet of aluminium foil 25 cm<sup>2</sup> (5 cm x 5 cm), itself held in position by a microporous hyperallergenic bandage 5 cm wide (Micropore), encircling the trunk of the animal without interfering with respiratory or abdominal movements.

. The maintenance and protection of this occlusive patch was ensured by a "sleeve" of elastic material.

- Length of exposure : 1 hour 30 minutes.

After removal of the patch, the surplus test article which did not penetrate was wiped with a gauze moistened with purified water.

- Irradiations :

. The guinea-pigs of group II were immobilised by a restraining system which allowed them to be subjected to irradiations ; however the posterior part of the back of each animal was covered with a sheet of aluminium foil in order to ensure that only the anterior part was exposed to the U.V. radiations. These irradiations were performed with the aid of a system of 2 fluorescent lamps having a continuous emission spectrum situated respectively at :

400 - 310 nm : black light fluorescent Mazdafluor lamp (Ref. TFWN 20 W - energetic flux released per square cm = 1.32 mW - spectral peak at 360 nm)

350 - 285 nm : solar fluorescent Westinghouse lamp (Réf. FS 20 T 12 - energetic flux released per square cm = 1.32 mW - spectral peak at 310 nm)

. These two lamps were placed 10 cm away from the backs of the guinea-pigs which were irradiated uniformly in groups of 11 with the aid of reflectors, for 5 minutes (energy released : 0.43 Joule/cm<sup>2</sup>). Moreover, in order to be under the same conditions as these employed for the "challenge" application which was performed at the end of this study, the animals were again irradiated but with 2 black light fluorescent Mazdafluor lamps only, placed at a distance of 5 cm for 1 hour 30 minutes (energy released : 12 Joules/cm<sup>2</sup>). The total energy liberated was thus 12.5 Joules/cm<sup>2</sup> and the level of U.V.B. radiation, 1 %:

. These lamps emitted mainly U.V.A. (wavelengths between 400 nm and 315 nm) which are the pigmentogenic rays and U.V.B. (wavelengths between 315 nm and 290 nm) which are responsible for solar erythema. The intensity and duration of the U.V.B. irradiation as well as the distance between the skin of the animal and the U.V.B. lamp were chosen to provoke a slight erythema (hardly visible : score = 1), approximately 6 hours after irradiation : this irritation which corresponds to the Minimal Erythematous Dose level (M.E.D.) has disappeared 24 hours later.

N.B. : the phototoxic reaction is essentially associated with short solar spectrum irradiations less than approximately 360 nm. The U.V.C. rays (wavelengths = 280 to 185 nm) which are damaging to the cells are theoretically filtered out in the upper atmosphere.

## 8.1.2. Observations and examinations performed

-----  
- Readings intervals : 6 and 24 hours after irradiation.

- Evaluation of the cutaneous macroscopic reactions : erythema and oedema were evaluated for each group of animals according to the scale described previously (7.2.2.). Any other abnormality was also listed : thickening, dryness of the skin, etc...

- Histopathological examinations of the skin : as no macroscopic reaction was noted in all the guinea-pigs examined, no cutaneous biopsy was performed for histopathological examination.

## 8.1.3. Analysis of phototoxicity data

a) Interpretation of the phototoxicity reactions

-----  
In order to maximise this method, the methods of irradiation which were chosen led to a slight irritation (Minimal Erythematous Dose level) about 6 hours after this exposure to the U.V. radiations ; but in contrast with the phototoxicity reactions, this irritation disappeared within 24 hours.

Thus, the scores which are shown in the phototoxicity results tables correspond to the difference obtained between the treated area (under the occlusive patch) and irradiated and the area irradiated but not treated situated around the patch (M.E.D.)

- Macroscopic examination :

. Positive reaction :

if by contrast with the irradiated but not treated area, the animal of group II (treated and irradiated) had a score for erythema and/or oedema equal to or greater than 2 in comparison with the scores attributed to group I guinea-pigs (treated but not irradiated) ;

. Negative reaction :

when the group II animal obtained a score of 0 or a score of 1 equal to that of the group I guinea-pigs.

. doubtful reaction :

any other cases.

- Histopathological examination :

The only sections of skin which were counted as positive were those showing images of "burn" reaction.

b) Expression of the phototoxicity results

The macroscopic readings and the histopathological examinations were performed blind :

- . the reaction was "positive", if the animal presented with a macroscopic cutaneous lesion which histology confirmed as a true phototoxicity reaction,
- . the reaction was "negative", if the animal did not present with a macroscopic abnormality or if histology could not confirm the macroscopic reaction,
- . the reaction was "doubtful", if a macroscopic lesion was noted for which the origin of the lesion observed could not be determined by histopathology.

N.B. : this part of the protocol where the application of the test article and irradiation were performed only once, just showed evidence of the phototoxic potential.

8.2. Evaluation of photoallergic potential

As the test article did not provoke any reaction of phototoxicity, this study was completed by the investigation of any possible photoallergic potential by continuing applications of the test article in the same animals under the same experimental conditions.

8.2.1. Experimental designa) Induction

- \* Intradermal injections of Freund's complete adjuvant :
  - Area of injection : retroscapular region into both sides of the site of application of the test article.
  - Time of administration : Day 4 (Day 2 : depilation with wax).
  - Dose level : 4 intradermal injections with 1 ml sterile syringe of 0.1 ml each of Freund's adjuvant diluted to 50 % (V/V) in an injectable isotonic solution (0.9 % NaCl).
  
- \* Topical applications of the test article :
  - Area of application : within the area delimited by the 4 intradermal injections of Freund's adjuvant.
  - Time of administration : Days 4, 7 and 9.
  - Dose level : 0.33 g (quantity corresponding to a volume of 0.5 ml) per animal of the test article as supplied.
  - Reason for the choice of the dose level : it is the maximum dose level which may be applied under the occlusive patch.
  - Methods of administration : to the previously depilated (Day 2) skin of all the animals (Groups I and II), for 1 hour 30 minutes under the occlusive patch previously described (8.1.1.).

- Irradiations (Group II only) : after removal of the patches corresponding to each one of the 3 applications, the guinea-pigs were irradiated by means of the two fluorescent lamps described previously, placed at a distance of 5 cm from the backs of the guinea-pigs for 15 minutes (energy released : 1.7 Joules/cm<sup>2</sup>), then with 2 black light Mazdafluor fluorescent lamps for 40 minutes at a distance of 5 cm (energy released : 5.4 Joules/cm<sup>2</sup>). For each irradiation the total energy liberated was thus 7.1 Joules/cm<sup>2</sup> and the level of U.V.B. radiation, 6 %.

b) Rest period

The animals received no treatment from Days 9 to 23, i.e. for a period of 14 days.

c) Challenge application

- Area of application : sacro-lumbar region, not previously treated or irradiated.

- Time of administration : Day 23 (Day 22 : clipping and depilation with cream).

- Dose level : 0.33 g (quantity corresponding to a volume of 0.5 ml) per animal of the test article as supplied (maximum non-irritant dose level determined at the time of the preliminary study).

- Methods of administration : to the clipped and depilated skin of all the animals (groups I and II), under the same conditions and under the occlusive patch described previously (8.1.1.).

- Length of exposure : 1 hour 30 minutes.

- Irradiations (group II only) : after removal of the patches the guinea-pigs were irradiated by means of the two black light Mazdafluor fluorescent lamps emitting between 400 and 310 nm (non-erythematous zone) and placed at a distance of 5 cm for 1 hour 30 minutes (total energy released : 12 Joules/cm<sup>2</sup>). In fact, it is the U.V.A. rays (400 to 315 nm) which are largely responsible for photoallergic "accidents", as only these rays are capable of damaging the mucous Malpighian bodies under natural conditions.

N.B. : tests performed on guinea-pigs of the same strain and age showed that this irradiation technique did not provoke a cutaneous reaction under the same experimental conditions.

8.2.2. Observations and examinations performed for the "challenge" application  
-----

- Reading intervals : 6, 24 and 48 hours after irradiation.

- Evaluation of the cutaneous macroscopic reactions : the erythema and oedema were evaluated for each group of animals according to the scales described previously (7.2.2.). Any other abnormality was similarly noted : vesicles, thickening, dryness of the skin, etc...

- Histopathological examinations : as no macroscopic reaction was noted in all the guinea-pigs examined, no cutaneous biopsy was performed for histopathological examination.

8.2.3. Analysis of photoallergy data

-----  
a) Interpretation of photoallergy reactions

- Macroscopic examination :

. Positive reaction :

when the group II animal (treated and irradiated) presented with, after the "challenge" application and irradiation, a clearly defined erythema and/or oedema, which score is equal to or greater than 2 in comparison with that obtained at the time of the 1st application, and with that of the group I animals (treated but not irradiated) after the final application ;

. Negative reaction :

when the group II animal presented with no abnormality after the "challenge" application and irradiation (score = 0), or if it presented with a reaction which was hardly visible (score = 1), identical to that obtained at the time of the 1st application, and that seen in the group I animals after the final application ;

. doubtful reaction :

any other cases.

- Histopathological examination :

The only sections of skin which were counted as positive were those showing images of experimental eczema.

b) Expression of the photoallergy results

The macroscopic readings and the histopathological examinations were performed blind :

. the reaction was "positive", if the animal presented with a cutaneous macroscopic lesion which histology confirmed as a true photoallergy reaction,

. the reaction was "negative", if the animal did not present with any cutaneous macroscopic lesion or if histology did not confirm the macroscopic reaction.

. the reaction was "doubtful", if a macroscopic lesion was noted for which the origin could not be determined by histopathology.

As a function of the number of positive reactions, the photoallergic potential of the test article in the skin of albino guinea-pigs was expressed in the following way :

PERCENTAGE (%) OF PHOTSENSITIZED ANIMALS	GRADE	PHOTOSENSITIZING POTENTIAL
>0 and <10	I	Weak *
11 to 25	II	Mild
26 to 50	III	Moderate
51 to 75	IV	Strong
76 to 100	V	Extreme

\* regarded as non-significant

Note : this study for the evaluation of the experimental photoallergic potential in the guinea-pig by the enclosed epicutaneous route, constitutes an evaluation of the probability of the photosensitizing potential of a substance. The extrapolation of the results to man is only valid within certain limits.

The only generalisation which can be made is that substances which are highly photosensitizing in the guinea-pig also provoke a large number of photosensitizing reactions in man, whilst substances which are weakly photosensitizing in the guinea-pig may or may not provoke photoallergic reactions in man.

#### 9. DATA RECORDING AND ARCHIVING

All the observations were recorded directly onto computer systems and simultaneously recorded on printed documents which were then considered as raw data.

The original documents, including the final report and all raw data, are kept in the archives of HAZLETON FRANCE for 10 years starting one month after the dispatch of the final report.

The test article is stored in our testing facility for 2 months after the dispatch of the final report. Then it is destroyed.

PROTOCOL ADHERENCE

. Slight variations of temperature were noted beyond the norms ( $20 \pm 3^{\circ}\text{C}$ ) with a maximum at  $23.5^{\circ}\text{C}$ .

. Variations of humidity were noted beyond the norms (30 to 70 % R.H.) with a maximum at 76 % R.H.

During the preliminary study, the gauze pad was moistened with sterile Codex liquid paraffin instead of purified water.

Taking into account the death observed in group II, a male was replaced by a female ; therefore there were 9 males and 11 females at the end of the test (instead of 10 males and 10 females).

These deviations were not considered to have affected the objective or the outcome of the study.

RESULTS

All the results of this study are reported in the following pages.

The sensitivity of this method was evaluated in our laboratory using different substances well known for their phototoxic and/or photoallergic potential (or their innocuity). The results obtained are presented in the appendix.

## RESULTS OF THE PRELIMINARY STUDY OF THE CUTANEOUS PHOTOTOXICITY AND PHOTOALLERGY TESTS IN THE GUINEA-PIG

TEST ARTICLE : UVINUL T 150 (Batch 19-0518)

APPLICATION : 0.33 g and 0.17 g (quantities corresponding to volumes of 0.50 and 0.25 ml) per animal of the test article as supplied.

DATE OF APPLICATION : 14/02/91

		EVALUATION OF REACTIONS AT DIFFERENT OBSERVATION TIMES						
		6 HOURS		24 HOURS		48 HOURS		
		AFTER REMOVAL OF PATCH-TESTS						
SEX	GUINEA-PIG No	Concentrations or doses	0.33 g	0.17 g	0.33 g	0.17 g	0.33 g	0.17 g
M	44877	Erythema	0	0	0	0	0	0
		Oedema	0	0	0	0	0	0
M	44878	Erythema	0	0	0	0	0	0
		Oedema	0	0	0	0	0	0
F	44879	Erythema	0	0	0	0	0	0
		Oedema	0	0	0	0	0	0
F	44880	Erythema	0	0	0	0	0	0
		Oedema	0	0	0	0	0	0

(M = Male - F = Female)

OBSERVATIONS: No abnormality was noted

DISCUSSION AND CONCLUSION OF THE PRELIMINARY STUDY

The test article applied as supplied and at the dose level of 0.33 g and 0.17 g (quantities corresponding to volumes of 0.50 and 0.25 ml) per animal did not provoke any macroscopic reaction of cutaneous intolerance.

The main study of the test to evaluate the phototoxic and photoallergic potentials by topical applications in the guinea-pig, was thus performed with the test article applied as supplied and at the dose level of 0.33 g per animal.

## RESULTS OF THE CUTANEOUS PHOTOTOXICITY AND PHOTOALLERGY TESTS IN THE GUINEA-PIG

TEST ARTICLE : UVINUL T 150 (Batch 19-0518)

APPLICATION : 0.33 g (quantity corresponding to a volume of 0.5 ml)  
of the test article as supplied.

DATE OF APPLICATION : 26/02/91

## GROUP I : TREATED BUT NON-IRRADIATED ANIMALS

SEX	GUINEA-PIGS No	PHOTOTOXICITY					PHOTOALLERGY					
		Day 1	Day 2	EVALUATION			Day 23	Day 24	Day 25	EVALUATION		
		READINGS MADE		REACTION		CONCLUSION	READINGS MADE			REACTION		CONCLUSION
		6 H	24 H	+	-		?	6 H	24 H	48 H	+	
after 1st irradiation of group II		Macro.	Histo.	after 5th CHALLENGE irradiation of group II			Macro.	Histo.				
M	Erythema	0	0	/	/	/	0	0	0	/	/	/
	Oedema	0	0				0	0	0			
M	Erythema	0	0	/	/	/	0	0	0	/	/	/
	Oedema	0	0				0	0	0			
M	Erythema	0	0	/	/	/	0	0	0	/	/	/
	Oedema	0	0				0	0	0			
F	Erythema	0	0	/	/	/	0	0	0	/	/	/
	Oedema	0	0				0	0	0			
F	Erythema	0	0	/	/	/	0	0	0	/	/	/
	Oedema	0	0				0	0	0			

(M = Male - F = Female - O = Yes - N = No)

OBSERVATIONS : See observation(s) on following page(s)

1 0 5 3 4 1

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OBSERVATION(S)

/ : Non applicable.

RESULTS OF THE CUTANEOUS PHOTOTOXICITY AND PHOTOALLERGY TESTS IN THE GUINEA-PIG

TEST ARTICLE : UVINUL T 150 (Batch 19-0518)

APPLICATION : 0.33 g (quantity corresponding to a volume of 0.5 ml)  
of the test article as supplied.

DATE OF APPLICATION : 26/02/91 GROUP II : TREATED AND IRRADIATED ANIMALS

X	GUINEA-PIGS No	PHOTOTOXICITY					PHOTOALLERGY					
		Day 1	Day 2	EVALUATION			Day 23	Day 24	Day 25	EVALUATION		
		READINGS MADE		REACTION		CONCLUSION	READINGS MADE			REACTION		CONCLUSION
		6 H	24 H	+	-		?	6 H	24 H	48 H	+	
after 1st irradiation		Macro.	Histo.	after 5th CHALLENGE irradiation			Macro.	Histo.				
M	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			
M	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			
M	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			
M	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			
M	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			
M	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			
M	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			
M	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			
F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			
M	Erythema	0	0	-	/	N	0	0	0	-	/	N
	Oedema	0	0				0	0	0			

(M = Male - F = Female - O = Yes - N = No)

OBSERVATIONS : See observation(s) on following page(s)

## RESULTS OF THE CUTANEOUS PHOTOTOXICITY AND PHOTOALLERGY TESTS IN THE GUINEA-PIG

TEST ARTICLE : UVINUL T 150 (Batch 19-0518)

APPLICATION : 0.33 g (quantity corresponding to a volume of 0.5 ml)  
of the test article as supplied.

DATE OF APPLICATION : 26/02/91 GROUP II : TREATED AND IRRADIATED ANIMALS

X	GUINEA-PIGS No	PHOTOTOXICITY					PHOTOALLERGY						
		Day 1	Day 2	EVALUATION		CONCLUSION	Day 23	Day 24	Day 25	EVALUATION		CONCLUSION	
		READINGS MADE		REACTION + = positive - = negative ? = doubtful	Macro.		Histo.	READINGS MADE			REACTION + = positive - = negative ? = doubtful		Macro.
		6 H	24 H			6 H		24 H	48 H				
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44986	Oedema	0	0				0	0	0			
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44987	Oedema	0	0				0	0	0			
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44988	Oedema	0	0				0	0	0			
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44989	Oedema	0	0				0	0	0			
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44990	Oedema	0	0				0	0	0			
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44991	Oedema	0	0				0	0	0			
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44992	Oedema	0	0				0	0	0			
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44993	Oedema	0	0				0	0	0			
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44994	Oedema	0	0				0	0	0			
	F	Erythema	0	0	-	/	N	0	0	0	-	/	N
	44995	Oedema	0	0				0	0	0			

(M = Male - F = Female - O = Yes - N = No)

OBSERVATIONS : See observation(s) on following page(s)

## OBSERVATION (S)

The male guinea-pig n° 44984 was found dead on 09/03/91 (necropsy : no abnormality). It was replaced by the female n° 44997 of the complementary group. The male of the complementary group was also found dead during the study.

/ : Non applicable.

DISCUSSION AND CONCLUSION

All applications were performed with the test article applied as supplied and at dose level of 0.33 g per animal.

From the observations, no difference was observed between animals of group I (treated but non irradiated guinea-pigs) and those of group II (treated and irradiated guinea-pigs).

As a conclusion, from the results obtained under the experimental conditions employed, the test article did not provoke any reaction of phototoxicity and photoallergy in the 20 treated and irradiated guinea-pigs.

APPENDIX

# EVALUATION OF THE PHOTOTOXIC POTENTIAL BY TOPICAL APPLICATIONS, IN THE GUINEA-PIG

Results obtained with the protocol of  
GUILLOT (J.-P.) - MARTINI (M.-C.) - GONNET (J.-F.) - CONVERT (P.) - PETIT (J.-P.) - COTTE (J)\*  
with reference substances.

TEST SUBSTANCES	EXPERIMENTAL CONDITIONS	RESULTS WITH HISTOLOGY
		% of animals showing a photo-toxicity reaction
8-METHOXYPSORALEN	In solution at 0.05 ‰ (W/W) in ethanol at 99.5 % In solution at 0.01 ‰ (W/W) in ethanol at 99.5 %	70 10
5-METHOXYPSORALEN	In solution at 0.025 ‰ (W/W) in ethanol at 99.5 %	20
EXTRACT OF ANGÉLICA ROOTS	In solution at 4 % (V/V) in ethanol at 99.5 % In solution at 1.5 % (V/V) in ethanol at 99.5 %	100 0
7-ACETYL-1, 1, 3, 4, 4, 6-HEXAMETHYL TETRA-HYDRONAPHTALENE	In solution at 10 % (W/W) in ethanol at 99.5 %	50
RUE EXTRACT	In solution at 4 % (V/V) in ethanol at 99.5 % In solution at 1.5 % (V/V) in ethanol at 99.5 %	30 0
1, 3, 4, 6, 7, 8-HEXAHYDRO-4, 6, 6, 7, 8, 8-HEXAMETHYL CYCLOPENTA-GAMMA-2-BENZOPYRANE	In solution at 10 % (V/V) in ethanol at 99.5 %	25
BITHIONOL	In solution at 1 % (W/W) in acetone	10
4-ACETYL-1, 1-DIMETHYL-6-TER-BUTYLINDANE	In solution at 10 % (W/W) in ethanol at 99.5 %	0
5-ACETYL-1, 1, 2, 3, 3, 6-HEXAMETHYL INDANE	In solution at 10 % (W/W) in ethanol at 99.5 %	0
1, 1, 4, 4-TETRAMETHYL-6-ETHYL-7-ACETYL-1, 2, 3, 4-TETRAHYDRONAPHTALENE	In solution at 10 % (W/W) in ethanol at 99.5 %	0
CUMIN EXTRACT	In solution at 4 % (V/V) in ethanol at 99.5 %	0

\* *Parfums Cosmétiques Arômes. Avr. Mai 1983 - 50 - 49-63*  
*J. Toxicol. - Cut. & Ocular Toxicol 1985, 4 (2), 117-133*

## EVALUATION OF THE PHOTOALLERGIC POTENTIAL BY TOPICAL APPLICATIONS, IN THE GUINEA-PIG

*Results obtained with the protocol of  
GUILLOT (J.-P.) - MARTINI (M.-C.) - GONNET (J.-F.) - CONVERT (P.) - PETIT (J.-P.) - COTTE (J.)  
with reference substances.*

TEST SUBSTANCES	EXPERIMENTAL CONDITIONS		RESULTS WITH HISTOLOGY
	INDUCTION	CHALLENGE EXPOSURE (non-irritant concentration)	% of photo-sensitized animals
PROMETHAZINE	In solution at 10 % (W/W) in ethanol at 70 %	In solution at 5 % (W/W) in ethanol at 70 %	80
	In solution at 10 % (W/W) in ethanol at 70 %	In solution at 2 % (W/W) in ethanol at 70 %	45
MUSK AMBRETTE	In solution at 10 % (W/W) in acetone	In solution at 2 % (W/W) in acetone	25
TETRACHLORO-SALICYLANILIDE (TCSA)	In solution at 5 % (W/W) in acetone	In solution at 1 % (W/W) in acetone	20
BITHIONOL	In solution at 5 % (W/W) in acetone	In solution at 1 % (W/W) in acetone	20
3, 5, 4, TRIBROMO SALICYLANILIDE (TBS)	In solution at 5 % (W/W) in (50/50) mixing ethanol at 99.5 % and acetone	In solution at 1 % (W/W) in (50/50) mixing ethanol at 99.5 % and acetone	5
SULFANILAMIDE	In solution at 5 % (W/W) in acetone	In solution at 1 % (W/W) in acetone	0
6-METHYLCOUMARIN	In solution at 5 % (W/W) in ethanol at 99.5 %	In solution at 1 % (W/W) in ethanol at 99.5 %	0

\* *Parfums Cosmétiques Arômes. Avr. Mai 1983 - 50 - 49-63.  
J. Toxicol. - Cut. & Ocular Toxicol 1985, 4 (2), 117-133*

# DIAGRAMMATIC PRESENTATION OF THE PROCEDURE FOR THE EVALUATION OF THE PHOTOTOXIC AND PHOTOALLERGIC POTENTIALS BY TOPICAL APPLICATIONS IN THE ALBINO GUINEA-PIG

GUILLOT (J.-P.) - MARTINI (M.-C.) - GONNET (J.-F.) - LOQUERIE (J.-F.)  
CONVERT (P.) and COTTE (J.)

	<b>PHOTOTOXICITY</b> + Induction : <b>PHOTOALLERGY</b>	<b>PHOTOALLERGY</b> Challenge exposure
<b>Group I (treated but non irradiated) :</b> 3 males and 2 females	→ Test article + (adjuvant) ;	Test article
<b>Group II (treated and irradiated) :</b> 10 males and 10 females	→ Test article + Irradiation + (adjuvant) .	Test article + Irradiation

## PHOTOTOXICITY

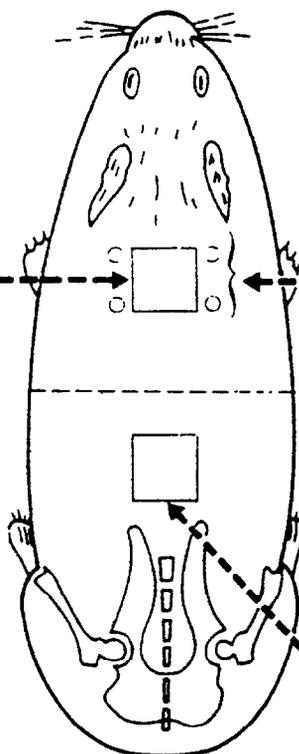
D-1 : Cream depilation.

D 1 :

— 1st occlusive topical application of 0.5 ml or g of the test article as supplied, or at the dose level or Maximum Non-Irritant Concentration (M.N.I.C.) to an area of 4 cm<sup>2</sup> for 90 mn.

— 1st irradiation (corresponding to the Minimal Erythematous Dose level) after removing the patch-tests : - 2 U.V. lamps (400-310 nm and 350-285 nm) → 5 mn, 10 cm from the back of the guinea-pigs ; - 1 U.V. lamp (400-310 nm) → 90 mn, 5 cm.

— Macroscopic and possibly histological examinations : 6 and 24 hours after the end of the irradiation.



GROUP II  
(Treated and Irradiated  
guinea-pig)

## PHOTOALLERGY

### INDUCTION

D 2 : Wax depilation.

D 4 :

— 4 intradermal injections of 0.1 ml of Freund complete adjuvant (diluted at 50 % in an isotonic injectable solution).  
— 2nd occlusive topical application of 0.5 ml or g of the test article as supplied, to an area of 4 cm<sup>2</sup> for 90 mn.  
— 2nd irradiation after removing the patch-tests : - 2 U.V. lamps (400-310 nm and 350-285 nm) → 15 mn, 5 cm from the back of the guinea-pigs ; - 1 U.V. lamp (400-310 nm) → 40 mn, 5 cm.

D 7 and D 9 : 3rd, 4th applications and irradiations carried out under the same conditions as previously described.

REST PERIOD : D 9 to D 23

### CHALLENGE EXPOSURE

D 22 : Cream depilation.

D 23 :

— Occlusive topical application of 0.5 ml or g of the test article as supplied or at the dose level or Maximum Non Irritant Concentration for 90 mn.  
— Irradiation after removing the patch-tests : - 1 U.V. lamps (400-310 nm) → 90 mn, 5 cm  
— Macroscopic and histological examinations : 6, 24 and 48 hours after the end of the irradiation.

TEST ARTICLE : UVINUL T 150 (Batch 19-0518)

Day 01            Day 23

## GROUP II : TREATED AND IRRADIATED GROUP

No 44976	399	575
No 44977	366	452
No 44978	346	414
No 44979	349	499
No 44980	329	459
No 44981	305	402
No 44982	353	458
No 44983	381	511
No 44984	342	Dead
No 44985	355	454

## GROUP I : TREATED BUT NON-IRRADIATED GROUP

No 44971	375	528
No 44972	358	464
No 44973	338	400

CUTANEOUS PHOTOTOXICITY AND PHOTOALLERGY TEST  
BODY WEIGHTS OF FEMALE GUINEA-PIGS (in grammes)

TEST ARTICLE : UVINUL T 150 (Batch 19-0518)

Day 01            Day 23

**GROUP II : TREATED AND IRRADIATED GROUP**

No 44986	398	491
No 44987	378	441
No 44988	361	423
No 44989	367	420
No 44990	356	446
No 44991	409	453
No 44992	373	422
No 44993	378	481
No 44994	355	437
No 44995	345	452

**COMPLEMENTARY GROUP**

No 44997	330	385
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**GROUP I : TREATED BUT NON-IRRADIATED GROUP**

No 44974	363	468
No 44975	349	458