THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

OPINION

CONCERNING

BENZOIC ACID, 2-[4-(DIETHYLAMINO)-2-HYDROXYBENZOYL]-, HEXYLESTER

Adopted by the SCCNFP during the 25th plenary meeting
of 20 October 2003

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

Request for inclusion of Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester in Annex VII, part 1 – List of permitted UV Filters which Cosmetic Products may contain – to Council Directive 76/768/EEC.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester safe for use in cosmetic products as a UV filter up to 10 %?

* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester

2.1.2. Synonyms

/

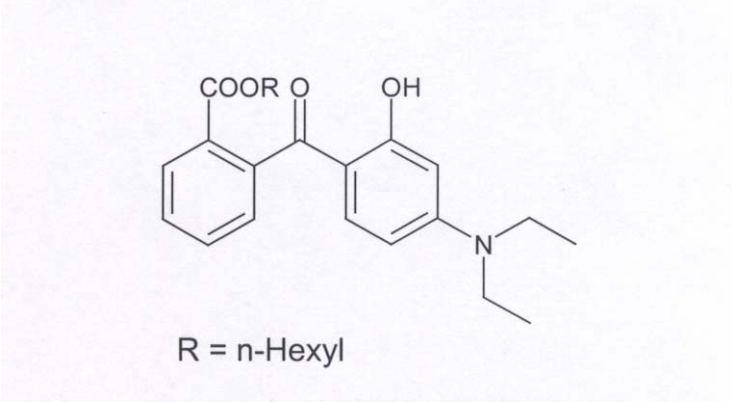
2.1.3. Trade names and abbreviations

Uvinul® A Plus

2.1.4. CAS no.

CAS n° : 302776-68-7
EINECS : /

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{24}H_{31}NO_4$
Mol weight : 397.52

2.1.7. Purity, composition and substance codes

Purity : 99.35%

The impurities were not addressed.

2.1.8. Physical properties

Appearance	:	nearly white fine-grained powder
Melting point	:	54 °C; 314 °C (decomposition temperature)
Boiling point	:	no boiling at normal pressure
Density	:	1.156 (D_4^{20})
Rel. vap. dens.	:	/
Vapour Press.	:	2.9 10^{-8} hPa ($p_{20°C}$); 7.9 10^{-7} hPa ($p_{50°C}$)
Log P_{ow}	:	6.2

2.1.9. Solubility

In water	:	< 0.01 mg/l at 20 °C and pH about 6-7
Receptor fluid*	:	1279 µg/ml (study 1)
		12 µg/ml (study 2)

* receptor fluid used in percutaneous absorption study 1 : ethanol/bi-distilled water, 1:1 v/v)
* receptor fluid used in percutaneous absorption study 2 : Krebs-Ringer bicarbonate buffer supplemented with 1% bovine serum albumin.

2.2. Function and uses

Requested use : up to 10% in sunscreen products alone or in combination with other UV absorbers.

Uvinul A plus is an oil soluble UVA filter that can be readily incorporated in the oil phase of emulsions. Due to its hydrophobic nature and its solubility in oil, it is particular suitable for water resistant formulations.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Method	:	According to OECD n° 423 (1996); EU n° B.1.tris (1196); US EPA, Health Effects Test Guidelines OPPTS 870.1100 ,Acute Oral Toxicity" (1998)
Test animals	:	6 Wistar rats (3 males/ 3 females)
Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch n°	:	R 323/681
Dosage	:	2000 mg/kg bw of the test material preparation in 0,5% Tylose CB 30.000 in Aqua bi-distillated

Observation : No abnormalities
LD_{50} : > 2000 mg/kg bw

Under the conditions of this study the median lethal dose of the test substance after oral dosing was found to be greater than 2000 mg/kg bw for the male and female animals.

Ref. : 1

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

No data

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

Sub-chronic Toxicity Study in Wistar Rats - Administration in the Diet for 3 Months

Method : According to OECD n° 408 (1998); EU n° B (1988)
Test substance : Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no : R 323/681
Animals & dose : 4 groups of 10 male and 4 groups of 10 female Wistar rats
group 0 : 0 ppm in the diet
group 1 : 600 ppm in the diet (males : approximately 51.7 mg/kg bw/day; females : approximately 59.3 mg/kg bw/day)
group 2 : 3,000 ppm in the diet (males: approximately 250.2 mg/kg bw/day; females: approximately 288.0 mg/kg bw/day)
group 3 : 15,000 ppm in the diet (males: approximately 1248.8 mg/kg bw/day females: approximately 1452.1 mg/kg bw/day)

Clinical examinations revealed no substance-related effects. All findings observed were spontaneous in nature. Clinical pathology also showed no substance-related effects.
The mean relative liver weights in male and female rats in high dose group were statistically significantly increased. However, the lack of any morphological changes supports the assumption that this is not an

adverse effect. Additionally, the absolute weights were not significantly decreased in either males (-3.6%) and females (-2.5%) in the high dose group.
All gross lesions and microscopic findings recorded were either single observations, or they occurred in control animals only, or they were recorded at low or comparable incidence and graded severity in control and high dose males and/or females. These changes are all considered to be unrelated to treatments. Comprehensive examinations of reproductive organs as well as sperm analysis did not give any indication for an impairment of fertility.

The no observed adverse effect level (NOAEL) under the conditions of this study was therefore 15,000 ppm (1248.8 mg/kg bw/day in males; 1452.1 mg/kg bw/day in females). Under conservative judgement, the NOEL was set at 3000 ppm (250 mg/kg bw).

Ref. : 11

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Method	:	According to OECD n° 404 (1992); EU n° B.5 (1992); US EPA, Health Effects Test Guidelines OPPTS 870.2400 "Acute Eye Irritation" (1998)
Test animals	:	3 White New Zealand Rabbits
Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no	:	R323/681
Dosage	:	A single topical application of 0.5 g to the intact skin for 4 hours under semiocclusive dressing

Slight erythema was observed in 2 animals on the day of application. No oedema was observed. The third animal did not show any skin reactions. The cutaneous reactions were reversible in the animals within 48 hours after removal of the patch at latest. The average score (24 to 72 hours) for irritation was calculated to be 0.1 for erythema and 0.0 for oedema.

Considering the observed cutaneous reactions as well as the average score for irritation, the test substance was not irritant to the skin under the test conditions.

Ref. : 2

2.4.2. Irritation (mucous membranes)

Method	:	According to OECD n° 405 (1987); EU n° B.5 (1992); US EPA, Health Effects Test Guidelines OPPTS 870.2400 "Acute Eye Irritation" (1998)
Test animals	:	3 White New Zealand rabbits
Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no	:	R323/681
Dosage	:	One single ocular application of 0.1 ml bulk volume (about 40 mg). 24 hours after application, the eye was rinsed with tap water.

Slight to moderate conjunctival redness was observed in all animals on the day of application. Additionally, slight discharge was seen in 1 animal. The ocular reactions were reversible in all animals within 48 hours after application at latest. The average score (24 to 72 hours) for irritation was calculated to be 0.0 for corneal opacity, iris and chemosis and 0.3 for conjunctival redness.

Considering the observed ocular reactions as well as the average score for irritation, the test substance was not irritant to the eye under the test conditions.

Ref. : 3

2.5. Sensitisation

Maximization Test in Guinea Pigs

Method	:	According to OECD n° 406 (1992); EU n° B.6 (1996); US EPA, Health Effects Test Guidelines OPPTS 870.2600 "Skin Sensitization" (1998); Japan MAFF guideline, 59 Noh San No. 4200, (1985)
Test animals	:	Guinea pigs
Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no	:	R323/681
Dosage	:	The following concentrations for induction and the challenge were selected on the basis of the pretests (intradermal and epicutaneous) :
Intradermal induction	:	test substance 5% in olive oil or 5% in Freund's adjuvant 10.9% aqueous NaCl-solution (1:1)
Epicutaneous induction	:	test substance 25 % in olive oil
Challenge	:	test substance 25 % in olive oil

Results
The intradermal induction with 5% test substance preparations caused moderate and confluent erythema and swelling or intense erythema and swelling in test group animals.
After the epicutaneous induction with a 25% test substance preparations incrustation, partially open (caused by the intradermal induction) could be observed in addition to moderate and confluent erythema and swelling in all test groups animals.
A challenge with a 25% test substance preparation in olive oil was performed 14 days after the epicutaneous induction. No skin reactions could be observed neither in control group 1 nor in the test group, 24 and 48 hours after removal of the patches. Olive oil, which was applied as a vehicle control to all animals, did not cause any skin reactions.
Since no borderline results were observed, a 2nd challenge was not performed.
It was concluded that the test substance does not have a sensitising effect on the skin of the guinea pig in the Maximization Test under the test conditions.

Ref. : 10

2.6. Teratogenicity

Prenatal Developmental Toxicity Study in Wistar Rats - Oral Administration (Gavage)

Method	:	According to OECD draft 414 (Draft 2000); EU n° B (1988); Japan/MHW: Guidelines for Toxicity Testing of Chemicals, Teratogenicity Test, MITI/MHW,1987 (Translation), pp. 212 - 213
Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no	:	R 323/681
Administration	:	as oily suspension by stomach tube (standard dose volume: 5ml/kg bw)
Duration	:	day 6 through day 19 post coitum (p.c.)
Groups and dose	:	3 groups of 25 mated female Wistar rats/group
		test group 1 : 40 mg/kg bw/day
		test group 2: 200 mg/kg bw/day
		test group 3: 1,000 mg/kg bw/day
		Control group : 25 females, dosed with the vehicle only (olive oil Ph.Eur./DAB)

Results
The oral administration to pregnant Wistar rats from implantation to one day prior to the expected day of parturition (days 6 - 19 p.c.) elicited some signs of maternal toxicity at 1,000 mg/kg bw/day. Maternal toxicity, by transient salivation, reduced food consumption on days 6 - 13 p.c. and slight impairments in absolute and corrected body weight gain was noted. No signs of substance-induced maternal toxicity occurred at dose levels of 40 or 200 mg/kg bw/day.
There were no substance-induced, dose related influences on the gestational parameters and no signs of prenatal developmental toxicity, especially no substance induced indications of teratogenicity, up to and including the highest dose level (1000 mg/kg bw/day).

The no observed adverse effect level (NOAEL) for maternal toxicity is 200-1000 mg/kg bw/day, while it is > 1000 mg/kg bw/day (highest applied dose) for prenatal developmental toxicity.

A comparison between the above-mentioned results and those derived from the 90-day study (NOAEL / NOEL) may be influenced by and due to the kind of administration (diet versus gavage of an oily suspension as bolus and consequently reaching actual higher systemic levels).

Ref. : 12

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous absorption

Study 1

Test substance	:	10 % a.i. in a cosmetic formulation (o/w emulsion, no composition stated). Solubility in receptor fluid is 1.28 mg/ml.
Batch n°	:	R323/681
Purity	:	99.35 %
Dosage	:	2 mg/cm² and 10 mg/cm²
Skin preparation	:	full-thickness pig skin. The method of skin preparation and the storage conditions of skin preparations were vaguely described
Skin temperature	:	32 ± 1 °C
Donor chamber	:	occlusion (covered with parafilm)
Receptor fluid	:	1:1 ethanol/water
Control	:	the vehicle (o/w emulsion in which the a.i. is incorporated) served as a control. No reference substance used.
Skin integrity	:	membrane integrity was visually checked prior to the test, not during the test.
Reproducibility	:	Overall recovery results (respectively 6 and 7 membranes /group) : Group 2 (2 mg/cm²) recoveries : Membrane : 5.99 to 21.42%, leading to 10.54 ± 5.59 % Receptor compt. : 0.13 to 1.54%, leading to 0.86 ± 0.46% Group 3 (10 mg/cm²) recoveries : Membrane : 2.62 to 12.54%, leading to 6.22 ± 4.23% Receptor compt. : 0.18 to 2.82%, leading to 1.05 ± 1.20%
Recovery	:	an overall recovery of 83 to 102 % accepted

Result
As it could be demonstrated by repeated extractions, the utmost amount of test substance was found in the donor compartment, but particularly in the membrane washings, followed by the epidermal membrane. Only 0.9% respectively 1.0% of the applied dose was found in the receptor compartment after the exposure period of 24h. Therefore, it can be assumed that most of the amount found in the epidermal membrane is located in the upper layers of the stratum corneum which will most probably not be absorbed.

Remarks
* 7 out of the 20 membranes had to be excluded from the study due to low recovery rates (below 80%) and/or due to leakage of receptor fluid on the upper side of the membrane.

* Tape stripping has not been performed in order to check the SC theory of the applicant. Viewing the fact that application of higher amounts of test substance induce higher amounts penetrated, it is not self-evident that this theory can be supported and that the amount in the SC can be ignored.
* The receptor fluid does not meet the demand and thus was regarded as inappropriate.

Conclusion
The percutaneous absorption study cannot be considered as valid due to the shortcomings mentioned above.

Ref. : 13

Study 2

Test substance	:	10% a.i. in a cosmetic formulation (o/w emulsion, no composition stated), solubility in receptor fluid = 12.353 µg/ml
Batch n°	:	30956/121D2 +/122D
Purity	:	97.9 %
Dosage	:	2 mg/cm² for 24 hours (finite dose scenario)
Skin preparation	:	Full-thickness pig skin (dermatomed skin) For the a.i. : 500 µm thickness For caffeine : 1000 µm thickness.
Skin temperature	:	32 °C
Donor chamber	:	No specification : occluded / unoccluded
Receptor fluid	:	Krebs-Ringer bicarbonate buffer supplemented with 1% bovine serum albumin. Solubility of a.i. = 12.353 µg/ml.
Control	:	A placebo test formulation was provided, but apparently not included in the test.
Skin integrity	:	Caffeine (10 mg/ml) in buffer was used as a marker compound, at 2 ml atop on the skin preparation (infinite dose scenario).
Recovery	:	Mean 92.7% ± 4.8%

Results
The percutaneous absorption study n° 2 can be considered as valid. The percutaneous absorption was set at 0.100 µg/cm² or 0.042%.

Ref. : 14

2.8. Mutagenicity/Genotoxicity

Bacterial Reverse Mutation Test

Method	:	According to OECD n° 471 (1997); EU n° B14 and B13 (1992)
Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester, batch no R323/681
Strains	:	*Salmonella typhimurium* TA 1535, TA 100, TA 1537, TA 98 and *Escherichia coli* WP2 uvrA
Dose range	:	Standard plate test : 20 µg - 5,000 µg/plate (in DMSO) Preincubation test : 4 µg - 2,500 µg/plate (in DMSO)
Test conditions	:	Standard plate test and preincubation test both with and without metabolic activation (Aroclor-induced rat liver S9-mix)
Solubility	:	Precipitation of the test substance was found from about 500 µg/plate onward.

An increase in the number of his+ or trp+ revertants was not observed in the standard plate test or in the preincubation test either without S9-mix or after the addition of a metabolizing system.
Conclusion
The test substance is not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions chosen.

Ref. : 4

***In vitro* Chromosome Aberration Assay in V79 Cells**

Method	:	According to OECD n° 473 (1997); EU n° B10 (1992)
Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no	:	R323/681
Cell system	:	V79 cell line derived from the Chinese hamster in MEM medium with glutamine supplemented with 10% foetal calf serum (not during exposure to the test substance), 1 % penicillin/streptomycin, 1 % amphotericine

Dose range : vehicle : DMSO

1St experiment
4 hours exposure, 18 hours harvest time, without S-9 mix : 0; 5.0; 10.0; 20.0 µg/ml
4 hours exposure, 18 hours harvest time, with S-9 mix : 0; 10.0; 20.0; 40.0 µg/ml
2nd experiment
18 hours exposure, 18 hours harvest time, without S-9 mix : 0; 2.5; 5.0; 10.0 µg/ml
18 hours exposure, 28 hours harvest time, without S-9 mix : 0; 10.0 µg/ml
4 hours exposure, 28 hours harvest time, with S-9 mix : 0; 10.0; 20.0; 40.0 µg/ml

Test conditions
About 2-3 hours prior to harvesting the cells, colcemid was added to arrest cells in a metaphase-like stage of mitosis (c-metaphases). After preparation of the chromosomes and staining with Giemsa, 100 metaphases for each culture in the case of the test substance and vehicle controls, or 50 cells for each culture in the case of the concurrent positive controls, were analyzed for chromosomal aberrations.

Result
The test substance did not cause any increase in the number of structurally aberrant metaphases incl. and excl. gaps at both sampling times either without S-9 mix or after adding a metabolizing system in two experiments performed independently of each other. No increase in the frequency of cells containing numerical aberrations was demonstrated either.
Conclusion
The test substance is considered not to be a chromosome-damaging (clastogenic) agent under in vitro conditions in V79 cells.

Ref. : 5

2.9. Carcinogenicity

No data

2.10. Special investigations

Chromosome Aberration Test *in vitro* : Photo-mutagenicity in Chinese Hamster V79 Cells

Method : According to SCC Guideline CSC/803-5/90 (1990) Guidelines for assessing the potential for toxicity of compounds used as sunscreen agents in

cosmetics, Annex 1, Notes for guidance for the toxicity testing of cosmetic ingredients; OECD n° 473 (1997); EU n° B 10 (2000)

Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no	:	R323/681,
Test system	:	Chinese Hamster V79 cell line
Dose range	:	2.5; 5.0; 10.0; 20.0; 40.0 and 80.0 µg/ml in DMSO
Irradiation		
Light source glass,	:	Xenon-lamp (Suntest CPS, ATLAS) with an additional special filter emitting visible and UVAIUVB light >290 nm
UV doses	:	225/11.25 mJ/cm^2 UVA/UVB (exp. I and II) or 375/18.75 mJ/cm^2 UVA/UVB (exp. II)
Positive controls	:	with irradiation : 8-Methoxypsoralene without irradiation : Ethylmethane sulfonate

Test conditions
The cultures were pre-incubated with the test substance for 30 min. After exposure to UV light and further 3 hours the cultures were washed twice. Corresponding cultures with the test substance were kept in the dark for 3 h exposure period. 18 hrs (exp. I) or 28 hrs (exp. II) after start of treatment, the cultures were prepared for cytogenetic evaluation. In the cytogenetic experiments for each experimental group two parallel cultures were set up. Per culture 100 metaphase plats were scored for structural chromosome aberrations.

Results
No biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed, neither in the absence nor in the presence of artificial sunlight.
No increase in the frequencies of polyploid metaphases was found after treatment with the test substance as compared to the frequencies of the controls.
Appropriate mutagens as positive controls induced statistically significant increases ($p< 0.05$) in cells with structural chromosome aberrations.

Conclusion
The test substance is considered to be non-photoclastogenic in this chromosomal aberration test.

Ref. : 6

Photomutagenicity in a *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay

Method	:	According to OECD n° 471 (1997); EU n° B14 and B13 (1992) SCC Guideline CSC/803-5/90 (1990)
Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no	:	R323/681
Strains	:	*Salmonella typhimurium* TA 1537, TA 98, TA 100, TA 102 and *Escherichia coli* WP2
Dose range	:	33; 100; 333; 1000; 2500; and 5000 µg/plate (in DMSO)
Test conditions	:	Source of light: Xenon-lamp (Suntest CPS, ATLAS) with a UV glass filter cutting off wave lengths below 290 nm

Toxicity

No toxic effects, evident as a reduction in the number of revertants or irregular background growth, were observed in the strains used.

Mutagenicity
No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance at any dose level. There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies. An irradiation specific positive control (8-methoxypsoralene) was used with strains TA 102 and WP2.

Conclusion
The test substance is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli photomutagenicity assay.

Ref. : 7

Phototoxic and Photoallergenic Potential by Cutaneous Route in Guinea Pigs

Method : The design of the study was based on the method published by Unkovic et al., Sci. Tech. Ani. Lab., 8, no 3: 149-160 (1983)
Test animals : Guinea pigs
Test substance : Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no : R323/681
Irradiation : An ultra-violet lamp „Toxicotronic" 312/365 nm (Vilbert/Lourmat) was used. The lamp consists of two groups of three fluorescent tubes producing either UV A(365 nm) or UV B (312nm). The irradiation was performed in two stages, first irradiation with UV B and then irradiation with UV A at an infra-erythematogenic irradiation dose (score of erythema ≤ 0.5). The irradiation doses were 9 joules/cm² for UV A and 0.1 joule/cm^2 for UV B.

Application route : Cutaneous. Cutaneous reactions were scored before and 1 hour, 4 and 24 hours after the single application and/or irradiation.

Treatment : Twenty-five animals were allocated to four groups :
Group 1(five animals) : irradiated control group
Group 2 (five animals) : group treated with the test substance
Group 3 (ten animals) : group treated with the test substance and irradiated
Group 4 (five animals) : vehicle control group

The phototoxic potential of the test substance was evaluated 1 hour, 4 and 24 hours after the first treatment and/or irradiation performed on day 1 in animals of all groups. The photoallergic potential of the test substance was assessed in animals of all groups after several treatments and/or irradiation during an induction period of 8 days on the anterior scapular area (6 applications - days 1 to 8), followed by a rest period of 20 days, then a challenge application and/or irridiation to the posterior area of the right (UV A) and left (UV B) flanks of the animals (day 29).
At each treatment, a dose-volume of 0.2 ml of the test substance at the concentration of 10 or 20% (w/w) in olive oil was applied by cutaneous route. A gentle massage was given to facilitate penetration of the test substance into the epidermis.

Results

No clinical signs and no deaths were noted during the study. The body weight gain of the treated animals was similar to that of the control animals.

Phototoxic potential
The cutaneous reactions observed on days 1 and 2 in almost all animals of groups 1,2,3 and 4 remained within the range of a local reaction at an infra-erythematogenic irradiation dose (questionable or weak erythema) and were of similar incidence in control and treated groups. No cutaneous reactions which could be attributed to a photoirritant effect of the test substance were observed.

Photoallergenic potential
The cutaneous reactions observed on day 29 in almost all animals of groups 1,2,3 and 4 remained within the range of a local reaction at an infra-erythematogenic irradiation dose (questionable or weak erythema) and were of similar incidence in control and treated groups. No cutaneous reactions which could be attributed to a photoallergenic effect of the test substance were observed.

Conclusion
Under the experimental conditions, topical applications of the test substance do not induce any phototoxic or photoallergenic reactions in guinea pigs.

Ref. : 8

Cytotoxicity Assay *in vitro* : Neutral Red (NR) Assay at simultaneous Irradiation with Artificial Sunlight

Method	:	EU n° B.41 (2000); OECD draft '*In vitro* 3T3 NRU phototoxicity test, (2000)
Test system	:	Balb/c 3T3 cells clone 31
Test substance	:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no	:	R323/681
Doses used	:	The test substance was dissolved in DMSO. The following concentrations were tested : 0.78; 1.56; 3.13; 6.25; 12.5; 25; 50 and 100 µg/ml
Treatment	:	Cytotoxicity was measured using the Neutral Red (NR) assay.

Results
No toxicity was observed in the absence of irradiation and only a slight toxicity was observed in the presence of irradiation with artificial sunlight. Therefore, only a ">PIF" value could be calculated. The EC_{50} value in the presence of irradiation (95 µg/ml) was determined graphically, the maximum tested concentration C_{max} in the absence of irradiation is 100 µg/ml, resulting in a >PIF of 1.05. This, however, is not biologically relevant in this case. No phototoxic potential can be predicted.
Conclusion
In the study described and under the experimental conditions reported no phototoxic potential was observed after treatment of Balb/c3T3 cells in the absence and in the presence of artificial sunlight.

Ref. : 9

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

(Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester)
(UV Filter)

Maximum absorption through the skin	**A (µg/cm²)**	**=**	**0.1 µg/cm²**
Typical body weight of human		**=**	**60 kg**
Skin Area Surface (whole body)	**SAS**	**=**	**18 000 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.800 mg**
Systemic exposure dose (SED)	**SAS x A x 0.001/ 60**	**=**	**0. 03 mg/kg**
No observed effect level (mg/kg) (rat, teratogenicity oral)	**NOAEL**	**=**	**200 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**6667**

2.12. Conclusions

Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester has low acute oral toxicity; more than 2000 mg/kg bw in the rat.
A NOEL, derived from an oral 90-day study in rats is about 1350 mg/kg bw and can be applied to a safety evaluation.
In a pre-natal development toxicity study, maternal toxicity was between 200-1000 mg/kg bw, obviously due to the kind of administration (gavage as bolus in oil), while > 1000 mg/kg bw can be regarded as NOEL for pre-natal development.

Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester is not irritating to the skin and mucous membranes in rabbits. It is not a dermal sensitiser.

The percutaneous absorption was set at 0.1 µg/cm².

Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester is neither phototoxic nor photosensitising. It is not mutagenic/photo-mutagenic *in vitro*.

As to a safety assessment for use of UV-filters by children over the age of 1 year, the SCCNFP issued a position statement (SCCNFP/0557/02).

2.13. Opinion

The SCCNFP is of the opinion that the use of benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester up to 10% in sunscreen products, alone or in combination with other UV absorbers, is safe.

2.14. References

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