EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

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

COLIPA n° S83

Adopted by the SCCP during the 8th plenary meeting
of 20 June 2006

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1. BACKGROUND

The Scientific Committee for Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted an opinion during the 25th plenary meeting of 20 October 2003 on Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-,hexylester, SCCNFP/0756/03 with the conclusion: "*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.*

Following this opinion the Commission has adopted a technical adaptation of the Cosmetic Directive by adding this UV-filter to the existing positive list of UV-filters (annex VII) by virtue of Directive 2005/9/EC.

According to the preamble to annex VII the authorised UV-filters "*may be added to other cosmetic products within the limits and under the conditions laid down in this annex*."

2. TERMS OF REFERENCE

1. *Does the SCCP consider that the use of the Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester is safe for the consumer in a concentration up to 10 % when used in other cosmetic products than sunscreen products?*

2. *Does the SCCP propose any further restrictions or conditions for its use in other cosmetic products?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

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

3.1.1.2. Chemical names

/

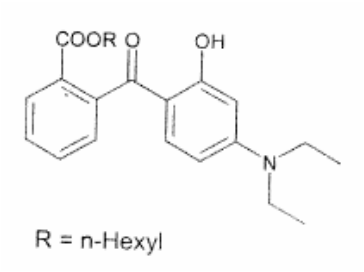
3.1.1.3. Trade names and abbreviations

Trade name: Uvinul® A Plus
COLIPA n°: S83

3.1.1.4. CAS / EINECS/ELINCS number

CAS : 302776-68-7
EILINCS : 443-860-6 (Uvinul A Plus)

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{24}H_{31}NO_4$

3.1.2. Physical form

Powder

3.1.3. Molecular weight

Molecular weight: 397.52

3.1.4. Purity, composition and substance codes

Purity: 99.35%

3.1.5. Impurities / accompanying contaminants

Impurities
Methanol 0.017g/100g
1-hexanol < 0.01g/100g
phthalic acid + phthalic anhydride < 0.01g/100g.

3.1.6. Solubility

In water: < 0.01 mg/l at 20 °C and pH about 6-7

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 6.2

3.1.8. Additional physical and chemical specifications

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. density:	/
Vapour Pressure:	2.9 10^{-8} hPa ($p_{20°C}$); 7.9 10^{-7} hPa ($p_{50°C}$)

3.1.9. Stability

The substance appears to be stable for 3 months at 25°C, 60% relative humidity and 40°C, 75% relative humidity

3.2. Function and uses

Up to 10% in sunscreen products alone or in combination with other UV absorbers.

Requested use: up to 10% when used in other cosmetic products than sunscreen products.

Uvinul® A Plus is an oil soluble UVA filter that can be readily incorporated in the oil phase of emulsions.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 423 (1996)
Species/strain:	Wistar rats
Group size:	3 males + 3 females (9 – 17 weeks old)
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R 323/681
Purity:	99.35%
Dose level:	2000 mg/kg bw in 0.5% Tylose CB 30.000 in Aqua bi-distillated
Route:	Oral, gavage, administration volume 10 ml
Observation:	14 days
GLP:	In compliance

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 rats.

Ref.: 1

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Acute

Guideline:	OECD 404 (1992)
Species/strain:	White New Zealand Rabbits
Group size:	3 young adult
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R 323/681
Purity:	99.35%
Dose level:	A single topical application of 0.5 g to the intact skin for 4 hours under semi-occlusive dressing
Route:	Topical
Exposure period:	4 hours
Observation:	72 hours
GLP:	in compliance

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

Repeated exposure

Guideline:	/
Species/strain:	Guinea pigs / Had Poc: DH (SPF)
Group size:	3 males and 3 females, 8 weeks old at start of experiment
Test substance:	Uvinul A Plus
Batch:	Labor Jr Nr. 31656/25-5
Purity:	98.8%
Dose level:	Daily applications with 50 µl of a 10% or 20% solution in propylene glycol for 14 days without use of dressing.
Route:	Topical
Exposure period:	14 days
Observation:	24 hours after application
GLP:	in compliance

There are no guidelines for the conduct of a 14-day skin irritation study available. The study was performed following the "Guidance for cosmetic safety evaluation" issued by the Japan Cosmetic Industry Association 2001, the EMEA/CPMP guidance document (The European Agency for the Evaluation of Medicinal Products, Committee for Proprietary Medicinal Products (CPMP): Note for guidance on non-clinical local tolerance testing of medicinal product. CPMP/SWP/2145/00: (March 2001) and the method of Marzulli and Maibach (Marzulli FN, Maibach HI. The rabbit as a model for evaluating skin irritants: a comparison of results obtained on animals and man using repeated skin exposures. Food & Cosmetic Toxicology, 13: 533-540, 1975).

The skin irritation of Uvinul A Plus in guinea pigs was examined by 14 open applications over a study period of 2 weeks. Two groups of 3 male and 3 female animals, each, were used and the test substance together with the negative control was tested on the right respectively left flank of the animals of a test group. Thus one test substance concentration and vehicle control were tested in each animal.
Amounts of 50 µl of test substance preparations in propylene glycol respectively the vehicle were applied to the intact skin in the flank region without use of dressing. Fourteen applications were performed daily over a study period of 2 weeks. The readings of skin reactions were performed 24 hours after each application.

Under the test conditions used in this study, the test substance concentrations did not cause skin reactions different from or discernibly more severe than those observed at the skin sites treated with the vehicle propylene glycol, alone. Furthermore no concentration response relation was present.

Ref.: 15

3.3.2.2. Mucous membrane irritation

Guideline:	OECD n° 405 (1987)
Species/strain:	White New Zealand Rabbits
Group size:	3 young adult
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch no:	R 323/681
Purity:	99.35%
Dose level:	One single ocular application of 0.1 ml bulk volume (about 40 mg). 24 hours after application, the eye was rinsed with tap water
Route:	Ocular application
Exposure period:	24 hours
Observation:	72 hours
GLP:	in compliance

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.

The test substance caused transient irritation of the eye under the test conditions.

Ref.: 3

3.3.3. Skin sensitisation

Maximization Test in Guinea Pigs

Guideline:	OECD 406 (1992)
Species/strain:	Guinea pigs
Group size:	10 animals in test group and 5 + 5 in control groups, young adult females (Bodyweight 327 – 375 g at beginning of study)
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R 323/681
Purity:	99.35%
Dose level:	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
Route:	Intradermal and epicutaneously occlusive
Exposure period:	24 hours
Observation:	72 hours
GLP:	In compliance

For intradermal induction, the test animals received 6 injections (2 injections of a 0.1 ml Freund's adjuvant/aqua dest 1:1, 2 injections of 0.1 ml of a 5% test substance formulation, 2 injections of a 0.1 ml 5% test substance formulation in Freund's adjuvant/aqua dest 1:1). The

intradermal induction with 5% test substance preparations caused moderate and confluent erythema and swelling or intense erythema and swelling in test group animals at 24 h after application.

Percutaneous induction was carried out 1 week after intradermal induction. The test substance (25% in olive oil) and the vehicle were applied for 48 h to the animals under occlusive conditions. Incrustation, erythema and oedema were observed in test and control animals at 48 h after beginning of application. After the epicutaneous induction, 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.

A positive control was not included in the study. Separate studies with a positive control (alpha-hexylcinnamaldehyde tech. 85%) are performed twice a year.

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

Comment
Several questions may be raised concerning the study. The study cannot be evaluated.

3.3.4. Dermal / percutaneous absorption

Study 1

Guideline:	OECD draft 428 (2000)
Test substance:	Cosmetic formulation (about 10% benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester) (o/w emulsion, no composition stated).
Batch:	R323/681
Purity:	99.35%
Dose applied:	2 mg/cm² and 10 mg/cm²; active substance 200 µg/cm^2 and 1 000 µg/cm^2
Skin preparation:	Full-thickness pig skin (epidermis and dermis). The method of skin preparation and the storage conditions of skin preparations were vaguely described
Skin temperature:	32 ± 1 °C
Exposure period:	24 h
Donor chamber:	Occlusion (covered with parafilm)
Receptor fluid:	1:1 Ethanol/water. Solubility in receptor fluid is 1.28 mg/ml.
Control:	The vehicle 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:	Mean total recovery of 83 and 102 %
GLP:	In compliance

As it could be demonstrated by repeated extractions, most of test substance was found in the donor compartment, but particularly in the membrane washings, followed by the epidermal membrane. Only 0.9% (group 2) respectively 1.0% (group 3) of the applied dose was found in the receptor compartment after the exposure period of 24 h. Therefore, the applicant 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 stratum corneum 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.

Ref.: 13

Comment

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

Study 2

Guideline:	OECD draft 428 (2000)
Test substance:	Cosmetic formulation (10% benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester) (o/w emulsion, no composition stated). Solubility in receptor fluid is 1.24 mg/ml.
Batch:	30956/121D2 +/122D
Purity:	97.9%
Dose applied:	2 mg/cm² for 24 hours (finite dose scenario); active substance 200 µg/cm^2
Skin preparation:	Full-thickness pig skin (dermatomed skin) For the a.i.: 500 µm thickness For caffeine: 1000 µm thickness.
Skin temperature:	32°C
Exposure period:	24 h
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:	No control was used.

Skin integrity:	Caffeine (10 mg/ml) in buffer was used as a marker compound, at 2 ml on the skin preparation (infinite dose scenario).
Recovery:	Mean 92.7% ± 4.8%
GLP:	In compliance

Dermatomized porcine skin biopsies (ca. 500 µm) were mounted into Franz diffusion cells and incubated with the test formulation (2 mg/cm^2 skin; 0.2 mg/cm^2 benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester) for 24 h. The experiment was performed in triplicate using 3 different pigs. At the end of the permeation study, the skin biopsies were separated into stratum corneum layers and deeper skin by tape stripping and quantified for benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester. Samples of the receptor fluid (1% bovine serum albumin in Krebs Ringer bicarbonate buffer) were analysed in suitable intervals and at the end of the incubation period (sensitivity of detection not mentioned).

The mean recovery was 93%. No permeation of benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester through the skin biopsies into the receptor medium could be observed. A minor amount (0.77%) was absorbed in the upper layers of stratum corneum clearly graded from amounts within the deeper skin layers (0.100 ±0.115 µg/cm^2; 0.042 ± 0.050%; max value 0.310 µg/cm^2; 0.149%).

Ref.: 14

Comment
The percutaneous absorption study no. 2 can be considered as valid. The percutaneous absorption was 0.100 ±0.115 µg/cm^2 or 0.042 ± 0.050% (Maximum value 0.310 µg/cm^2 or 0.149%).

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated dose (28 days) oral / dermal / inhalation toxicity

No data submitted

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Oral

Guideline:	OECD 408 (1998)
Species/strain:	Wistar rats Crl: W1 (GLX/BRL/HAN) IGS BR
Group size:	10 animals per sex and dose (42 days old at start of study)
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R 323/681
Purity:	99.35%
Dose levels:	0, 600 ppm (males: approx 51.7 mg/kg bw/d; females: approx 59.3 mg/kg/d), 3,000 ppm (males: approx 250.2 mg/kg bw/d; females: approx 288.0 mg/kg bw/d), 15,000 ppm (males: approx. 1249 mg/kg bw/d; females: approx 1452 mg/kg bw/d)
Route:	Oral, in diet
Exposure period:	90 days
GLP:	In compliance

Clinical examinations revealed no substance-related effects. Clinical pathology also showed no substance-related effects.

The mean relative liver weights in male (+7%) and female rats (+10%) 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.

The mean relative weights of testes (+ 9%, high dose group) and heart (female, low dose group +15%) were significantly increased. Whereas the mean relative weight of the spleen (mid dose group in females) was significantly decreased (-31%), the applicant does not regard this as treatment related.

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 by the applicant. Comprehensive examinations of reproductive organs as well as sperm analysis did not give any indication for an impairment of fertility.

Ref.: 11

Comment
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/d in females). Based on the increase in relative liver weight (+7% in male rats), the NOEL was set at 3,000 ppm (250 mg/kg bw/d).

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline:	OECD 471
Species/strains:	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 uvrA
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R323/681
Purity:	99.35%
Replicates:	3 plates per test
Concentrations:	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 onwards
GLP:	In compliance

The test substance has been investigated for the induction of gene mutation in *Salmonella typhimurium* and *Escherichia coli.* Liver S9 fraction from Sprague Dawley induced with Aroclor 1254 was used as the exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline.

A slight decrease in the number of revertants was observed in the standard plate test depending on the strain and test conditions from about 500 µg – 2,500 µg/plate onward. In the preincubation assay a weak bacteriotoxic (slight decrease in the number of revertants and/or slight reduction in the titer) was observed depending on the strain and test conditions from about 100 µg – 500 µg/plate onward. Test substance precipitation was found from 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 or with S9-mix as metabolising system.

Ref.: 4

Comment
The test substance is not mutagenic in the *Salmonella typhimurium/Escherichia coli* reverse mutation assay under the experimental conditions used.

Chromosome Aberration Assay in V79 Cells

Guideline:	OECD 473
Species/strains:	V79 cells derived from Chinese Hamster
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R323/681
Purity:	99.35%
Cell system:	V79 cell line in MEM medium with glutamine supplemented with 10% foetal calf serum (not during exposure to the test substance), 1 % penicillin/streptomycin, 1 % amphotericine
Concentrations:	vehicle: DMSO 1st experiment 4 h exposure, 18 h harvest time, - S-9 mix: 0; 5.0; 10.0; 20.0 µg/ml 4 h exposure, 18 h harvest time, +S-9 mix: 0; 10.0; 20.0; 40.0 µg/ml 2nd experiment 18 h exposure, 18 h harvest time, -S-9 mix:0; 2.5; 5.0; 10.0 µg/ml 18 h exposure, 28 h harvest time, +S-9 mix:0; 10.0 µg/ml 4 h exposure, 28 h harvest time, +S-9 mix: 0; 10.0; 20.0; 40.0 µg/ml

The test substance has been investigated for the induction of chromosome aberrations in V79 cells. Liver S9 fraction from Sprague Dawley induced with Aroclor 1254 was used as the exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline

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.

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.

Ref.: 5

Comment
The test substance is considered not to be a chromosome-damaging (clastogenic) agent under *in vitro* conditions in V79 cells.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

No data submitted

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

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

Guideline:	OECD draft 414 (2000)
Species/strain:	Sexually mature, virgin Wistar rats (CRL:WI (GLX/BRL/HAN)IGS BR)
Group size:	25 mated rats
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester suspended in olive oil
Batch:	R 323/681
Purity:	99.35%
Dose level:	0, 40, 200, 1000 mg/kg bw/d
Route:	Oral (gavage), 5 ml/kg bw
Exposure period:	Day 6 – 19 p.c.
GLP:	in compliance

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 alterations in absolute and corrected body weight gain were noted. No signs of substance-induced maternal toxicity occurred at dose levels of 40 or 200 mg/kg bw/d.

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/d).

Ref.: 12

Comment
The no observed adverse effect level (NOAEL) for maternal toxicity is 200 mg/kg bw/d, 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 administration (diet versus gavage).

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

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

Guideline:	OECD draft '*In vitro* 3T3 NRU phototoxicity test, (2000) EU n° B.41 (2000)
Species/strain:	Balb/c 3T3 cells clone 31
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R323/681
Purity:	99.35%
Concentrations:	0, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 µg/ml. The test substance was dissolved in DMSO
Artificial sunlight:	Dr. Honle Sol 500 solar simulator. Wavelength of the solar simulator with the filter was > 320 nm. Dose: 1.7 mW/cm^2 (the U.V. intensity underneath the lid) for 50 min at room temperature (= 5 J/cm^2).
GLP:	In compliance

After 1 h pre-incubation with 8 concentrations of the test substance or the positive control (chloropromazine; 6.25 – 200 µg/ml without irradiation and 0.125 – 40 µg/ml with irradiation), the cells were irradiated with artificial sunlight. Parallel cultures were kept in the dark. The cytotoxic response curves of the test groups were compared. The EC_{50}-values were determined and compared to calculate a photo-irritancy factor (PIF) to measure a possible phototoxicity.

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.

Ref.: 9

Comment
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.

Phototoxic and Photoallergenic Potential by Cutaneous Route in Guinea Pigs

Guideline:	/
Species/strain:	Dunkin-Hartley guinea pigs, male
Group size:	5 or 10 animals per group
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R323/681
Purity:	99.35%
UV irradiation:	Toxicotronic 312/365 nm (Vilbert/Lourmat). The lamp consists of two groups of three fluorescent tubes producing either UVA (365 nm) or UVB (312nm). The irradiation was performed in two stages, first irradiation with UVB and then irradiation with UVA at an infra-erythematogenic irradiation dose (score of erythema $\leq$ 0.5). The irradiation doses were 9 joules/cm² for UVA and 0.1 joule/cm^2 for UVB.
Dose levels:	0.2 ml of the test substance at the concentration of 10 or 20% (w/w) in olive oil
Groups:	Group 1(5 animals): irradiated control group Group 2 (5 animals): group treated with the test substance Group 3 (10 animals): group treated with the test substance and irradiated Group 4 (5 animals): vehicle control group
Route:	Topical
Observation period:	1, 4, and 24 hours after the single application and/or irradiation.
GLP:	In compliance

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). The experiments were performed in the period 13/12/2000 to 12/1/2001.

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 photoallergenic 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 irradiation to the posterior area of the right (UVA) and left (UVB) flanks of the animals. 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. The irradiation dose of UVA and UVB was infra-erythematogenic. The cutaneous reactions were evaluated at the treatment site.

At the end of the study, animals were killed without examination of internal organs. Skin samples were taken from the challenge application sites of the animals showing skin reactions at the last observation. No histological examination was performed.

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.

<u>Phototoxic potential</u>

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.

Ref.: 8

Comment

Under the experimental conditions, two very specific wavelengths of UV radiation were used without information of the absorption spectra of the substance. Broadband UVA and UVB irradiation would more appropriate mimicked the intended use of this cosmetic UV-filter.

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

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

Guideline:	OECD 471 (1997)
Species/strains:	*Salmonella typhimurium* TA98, TA100, TA102, TA 1537 and *Escherichia coli* WP2
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R323/681
Purity:	99.35%
Replicates:	3 plates per test
Concentrations:	33 – 5000 µg/plate (in DMSO)
UV irradiation:	Source of light: Xenon-lamp (Suntest CPS, ATLAS) with a UV glass filter cutting off wave lengths below 290 nm. UV dose was chosen that increased the number of revertant colonies to approximately twice the number of spontaneous revertants without irradiation. (TA1537: 50 mJ/cm^2 UVA, 2.5 mJ/cm^2 UVB; TA98: 20 mJ/cm^2 UVA, 1.0 mJ/cm^2 UVB; TA100: 4 mJ/cm^2 UVA, 0.2 mJ/cm^2 UVB; TA102: 100 mJ/cm^2 UVA, 5.0 mJ/cm^2 UVB; and WP2: 9 mJ/cm^2 UVA, 0.28 mJ/cm^2 UVB)
GLP:	In compliance

This study was performed to investigate the potential of benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester to induce gene mutations under irradiation with artificial sunlight according to the plate incorporation test (experiment I) and the preincubation test (experiment II) using the *Salmonella typhimurium* strains TA1537, TA98, TA100, TA102, and the *Escherichia coli* strain WP2. The assay was performed in two independent experiments. Each concentration and the controls were tested in triplicate.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester 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. In experiment II (pre-incubation), the numbers of revertants of the irradiated bacteria are not always twice as high as compared to the non irradiated control. In contrast to the plate incorporation assay used in the first experiment, the bacteria are not irradiated at or close to the surface during preincubation in aqueous solution. In this design UV light may be partially absorbed by the solution prior to reaching the bacteria reducing the direct DNA damage. However since the colony count of the positive control clearly exceeded the threshold of twice the colony count of the corresponding solvent control the data are judged as valid. 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 only used with strains TA 102 and WP2. The performance of the other strains was ensured with conventional positive controls in the absence of irradiation.

In conclusion, it can be stated that during the described photomutagenicity test and under the experimental conditions reported, the test substance did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Ref.: 7

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

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

Guideline:	OECD n° 473 (1997)
Species/strains:	V79 cells derived from Chinese Hamster
Test substance:	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester
Batch:	R323/681
Purity:	99.35%
Concentrations:	2.5; 5.0; 10.0; 20.0; 40.0 and 80.0 µg/ml in DMSO
Cell system:	V79 cell line in MEM medium with glutamine supplemented with 10% foetal calf serum (not during exposure to the test substance), 1 % penicillin/streptomycin, 1 % amphotericine
Light source:	Xenon-lamp (Suntest CPS, ATLAS) with an additional special filter glass, emitting visible and UVA/UVB 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

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. 100 metaphase per culture were scored for structural chromosome aberrations.

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.

Ref.: 6

Comment
Under the experimental conditions reported the test substance, benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester, was non-clastogenic in the absence and presence of irradiation in the *in vitro* chromosome aberration assay using the Chinese Hamster V79 cell line.

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

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

The safety calculation is only considering dermal exposure.

Maximum dermal absorption of test substance reported was 0.310 µg/cm²

Maximum absorption through the skin	DA_a (µg/cm²)	=	0.31 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 0.00031	=	5.83 mg
Systemic exposure dose (SED)	SAS x A x 0.001/ 60	=	0.093 mg/kg
No observed adverse effect level (mg/kg) (rat, teratogenicity oral, maternal toxicity)	NOAEL	=	200 mg/kg

Margin of Safety	**NOAEL / SED**	**=**	**2150**

3.3.14. Discussion

The safety has only been considered for dermal exposure.

If it is intended that it should be widely used, the environmental aspects should be considered.

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 was about 250 mg/kg bw/d. In a prenatal development toxicity study, the NOAEL for maternal toxicity was 200 mg/kg bw/d and 1000 mg/kg bw for prenatal developmental toxicity.

Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester is not irritating to the skin of guinea pigs for treatments up to 14 days. It caused transient irritation to the rabbit eye. A study of skin sensitisation in guinea pigs cannot be evaluated.

The percutaneous absorption was 0.100 ± 0.115 µg/cm^2 or 0.042 ± 0.050% (Maximum value: 0.310 µg/cm^2 or 0.149%).

Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]-, hexylester did not induce mutations in a bacteria test or chromosome aberration in V79 cells in the dark or under irradiation with artificial sunlight. It is neither phototoxic nor photosensitising.

No data on possible carcinogenic effect has been presented.

4. CONCLUSION

Although this substance is presently permitted and used as a sunscreen, the SCCP is of the opinion that the information submitted is not conform to current standards and guidelines for the safety evaluation of cosmetic ingredients.

Before any further consideration, the following information is required:

- an absorbance spectrum of the substance
- a mammalian gene mutation test.

The applicant should specify for what other purposes the substance should be used.

5. MINORITY OPINION

Not applicable

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7. ACKNOWLEDGEMENTS

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