EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

OPINION ON

BENZOPHENONE-3

COLIPA N° S38

Opinion adopted by the SCCP during the 10th plenary of 19 December 2006

ACKNOWLEDGEMENTS

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Keywords: SCCP, scientific opinion, UV filters, benzophenone-3, S38, Directive 768/76/EEC, CAS 131-57-7

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1. BACKGROUND

Submission I on the UV-filter Oxybenzone (the INN name), also known as Benzophenone-3 or 2-hydroxy-4-methoxybenzone, has been submitted by COLIPA[1].

Benzophenone-3 is proposed to be continued for use in sunscreen products at a maximum concentration at 10% weight/weight.

The substance is currently regulated in the cosmetics directive in annex VII, part 1 list of permitted UV filters which cosmetic product may contain. The regulation demands a warning on the label "contains oxybenzone".

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 the use of 2-hydroxy-4-methoxybenzone in a concentration up to 10% w/w in sunscreen products safe for the consumer?*

2. *Does the SCCP consider the use of 2-hydroxy-4-methoxybenzone in a concentration up to 10% w/w in other products than sunscreen products safe for the consumer?*

3. *Does the SCCP foresee any other restrictions to the safe use of 2-hydroxy-4-methoxybenzone?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1. Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI name: Benzophenone-3

Ref.: 4, 5, 6, 46, 66

3.1.1.2 Chemical names

Oxybenzone (INN name)
2-hydroxy-4-methoxybenzophenone
(2-Hydroxy-4-methoxyphenyl)phenyl methanone
2-Benzoyl-5-methoxyphenol

Ref.: 46, 66

3.1.1.3 Trade names and abbreviations

Aduvex 24 Escalol 567 Seesorb 101

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

Advastab 45	Eusolex® 4360	Spectra-Sorb UV 9
Anuvex	MOB	Sunscreen UV 15
ASL 24	Neo Heliopan BB	Uvasorb MET/C
Chimassorb 90	NSC 7778	Uvinul® M 40
Cyasorb UV 9	Ongrostab HMB	Uvistat 24
Cyasorb UV 9 Light Absorber	Onzone	Viosorb 110

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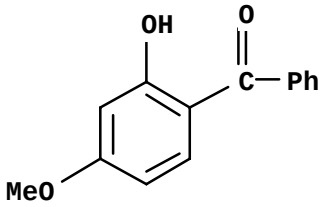
Ref.: 46

3.1.1.4 CAS / EINECS number

CAS: 131-57-7
EINECS: 205-031-5

Ref.: 4, 5, 6, 46, 66

3.1.1.5 Structural formula



3.1.1.6. Empirical formula

Molecular formula: C14H12O3

Ref.: 4, 5, 6, 46, 66

3.1.2 . Physical form

White yellowish, cream coloured powder

Ref.: 46

3.1.3 . Molecular weight

228.26 g/mol

Ref.: 5, 6, 46, 63, 65, 66

3.1.4 . Purity, composition and substance codes

Assay (GC): ≥ 99%*
IR-spectrum: conform**
UV-spectrum: conform**

Ref.: 4, 5, 6, 46

* Capillary Gas Chromatography, chromatogram available, batch nr. stated; no identification of 3 impurities at 0.1%)
** Just a mention in a Technical Data Sheet or Material Safety Data Sheet, no full description of test (standard UV-spectrum available, without batch nr. tested).

3.1.5 Impurities / accompanying contaminants

Organic solvents:	< 0.01% Xylene
Polycyclic aromatic hydrocarbons:	< 10 ppb (total)
Benzo(a)-pyrene:	< 1 ppb
Heavy metals:	< 10 ppm (guaranteed for all batches, with corresponding limits) [46]

3.1.6 Solubility

Water:	0.0037 g/l (20°C)
Glycerin:	< 0.01%
Abil® AV 8853:	2.0%
Jojoba oil:	6.0%
Ethanol:	6.0%
Isostearyl stearate:	7.0%
Isostearyl neopentanoate:	8.0%
Olive oil:	9.0%
Peanut oil:	9.0%
Cetiol® V:	9.0%
Isopropyl stearate:	9.0%
Isopropanol, butanol:	10.0%
Isopropyl myristate:	11.0%
Miglyol® 812:	14.0%
Finsolv® TN:	15.0%
Cetiol® HE:	17.0%
Citroflex® 2:	> 20.0%
Aceton:	> 20.0%
Chloroform:	> 20.0%

Ref.: 5, 6, 46

Note
These values are taken out of Technical Data Sheets or Material Safety Data Sheets.

3.1.7. Partition coefficient (Log Pow)

> 3.7 (n-octanol/water)

Ref.: 46

Note
This value is taken out of a Material Safety Data Sheet.

3.1.8. Additional physical and chemical specifications

Melting point:	62° - 65°C
Solidification point:	62° - 65°C
Loss on drying (40° C):	< 2%
Relative density:	1.32 at 25°C
Ash content at 650°C:	0.1% (upper limit)
Colour number (Gardner):	< 4
K-Value:	64 – 67
Odour:	almost odourless or faint characteristic
Flash point:	> 100°C
Extinction (UV/VIS spectrum in methanol)	400 (0.10 mg/ml cuvette 0.1 cm)
Specific absorbance:	630 - 670 (at 287 nm; 1%, 1 cm, methanol)

Ref.: 5, 6, 46, 63, 65, 66

Note
These values are taken out of Technical Data Sheets or Material Safety Data Sheets.

3.1.9. Stability

Shelf life: at least 2 years
Stability in distilled water: at least 96 hours*
Stability in DMSO: at least 4 hours*
Stability in corn oil: at least 10 days**
Stability in acetone: at least 3 weeks***
Stability in oily lotion: at least 3 weeks***

* determined within recent photomutagenicity studies (full description of stability study under GLP available)
** determined within recent prenatal developmental toxicity study, performed under GLP
*** determined within the oral and dermal US National Toxicology Program (NTP) studies

Ref.: 6, 11, 15, 30, 46, 63

General comments on chemical and physical specifications

Although it is acknowledged that Benzophenone-3 has been used for many years in several types of applications and that the chemical and physical specifications have been extensively studied in the past, it is the opinion of the SCCP that characterization and determination of purity should be based upon raw data instead of simple mentions in material technical/safety data sheets. At least the solubility in water, partition coefficient and the chemical characterisation, including the UV-spectrum, should be given for a recently tested batch.

3.2 FUNCTION AND USES

Benzophenone-3 is used as a broad-band UV filter in concentrations of up to 10% in sunscreen products alone or in combination with other UV filters.

Beside the usage in sunscreens, Benzophenone-3 is incorporated in other types of cosmetic products at concentrations ranging between 0.05 - 0.5% for product protection (photoprotection).

Ref. : 5, 46, 66

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

A number of acute toxicity studies are briefly described and generated the following values :
LD_{50}-oral-rat > 6,000 mg/kg (1953)
LD_{50}-oral-rat = 11,600 mg/kg (1964)
LD_{50}-oral-rat > 12,800 mg/kg (1972)
LD_{50}-dermal-rabbit > 16,000 mg/kg (1953)

Comment
Due to their dates of execution, the studies were not performed according to the current guidelines and GLP practices. Nevertheless it does not appear appropriate from an ethical point of view to request more recent acute toxicity data on Benzophenone-3.

Ref.: 35, 44, 58

3.3.2. Irritation and corrosivity

3.3.2.1 Skin irritation - rabbit

In a report of 1965, Benzophenone-3 is declared to be non-irritant to the rabbit skin (tested in 6 rabbits, all scores remained 0) [59]. This finding is reinforced by a second report of 1976 [1].
A third report of 1979 mentions that 0.5 ml of a sunscreen called *Protective Eye Cream* was also tested on the skin of 3 rabbits and was found to be non-irritating (all scores = 0) [16].

Comment
These 3 reports were very short (1-2 pages) and provided limited data information.

Ref.: 1, 16, 59

3.3.2.2 Mucous membrane irritation - rabbit

In a report of 1965, Benzophenone-3 is declared to be non-irritant to the rabbit eye (tested in 6 animals, all scores remained 0) [60]. This finding was reinforced by two other reports, respectively from 1953 [35] and 1976 [1].
A report of 1979 mentions that 0.1 ml of a sunscreen called *Protective Eye Cream* was instilled in the eye of 3 rabbits and was found to be non-irritating (all scores = 0) [16].

Comment
Again the 4 available reports are very short (1-2 pages) and provide only limited data information.

Ref.: 1, 35, 16, 60

3.3.2.3. Overall conclusion on irritation

The available studies on the skin and mucous irritation potential of Benzophenone-3 are old and therefore not conform to current guideline requirements. Nevertheless they may provide some useful information, since more animals than currently required were used, and in the skin irritation studies the test substance was also applied to the abraded skin representing a worst case situation. Since all results are consistent with each other and taking into consideration the aspect of animal welfare, it does not seem appropriate to ask for new skin and/or eye irritation studies with Benzophenone-3. The compound was shown to have no irritating potential for the skin and eyes of rabbits.

3.3.3. Skin sensitisation

3.3.3.1. Magnusson Kligman Maximisation test - Guinea pig

Date of study:	July 1978
Guideline/method:	Maximization test according to Magnusson and Kligman (1969), precursor of Annex V to Dir. 67/548/EEC, Method B.6 and OECD Guideline 406
Species/strain:	Guinea pig/Hartley white
Group size:	30 female animals in the control, 20 female animals in the test groups
Test substance:	Uvinul M-40 (Benzophenone-3)
Dosage levels:	Induction: 10%, Challenge 2.5%
Route:	Intradermal (induction) and percutaneous (booster and challenge)
Batch:	Not stated

Purity:	Not stated
GLP:	Study performed prior to implementation of GLP

In a dose-range finding study concentrations of 0.25%, 2.5%, 5% and 10% of Benzophenone-3 in petrolatum and the pure compound were applied on the shaved flanks of the animals. Based on the results of this study the animals were intradermally injected with 0.05 ml of 5% Benzophenone-3 in corn oil or 5% Benzophenone-3 in 50% aqueous Freund's complete adjuvant into the shaven shoulder region of 10 animals. One week after the induction injection, a topical booster patch consisting of 0.1 ml of 10% test substance in petrolatum was occlusively applied on the induction site for 48 hours. Prior to the booster, 10% aqueous Sodium Lauryl Sulfate was applied unoccluded to the induction sites of all animals.
The challenge was performed by occlusive epicutaneous application of 0.1 ml of 2.5% test material in petrolatum to a previously untreated site for 24 hours. The application sites were scored 24 and 48 hours after removal of the patch.

At readings 48 hours after challenge 18/20 animals showed no evidence of any effect and 2/20 revealed a barely perceptible erythema. At the 72 hour readings 1/20 showed no skin reaction and 3/20 barely perceptible erythema (different animals to the first reading). No skin reactions were observed in the control group at challenge, while the animals of the positive control group (2% phenylacetaldehyde in petrolatum) showed clear skin reactions as indication of sensitization.

The study authors conclude that Benzophenone-3 did not exhibit any potential to induce dermal sensitization in the performed Guinea pig Magnusson Kligman Maximization test.

Ref.: 2

3.3.3.2 Local Lymph Node Assay - mouse

Date of study:	September 2005
Guideline/method:	Annex V to Dir. 67/548/EEC, Method B.42; OECD Guideline 429
Species/strain:	Mouse, CBA/CaOlaHsd
Group size:	4 female animals per treated and control group
Test substance:	Benzophenone-3
Batch:	101
Purity:	99.8% (GC-FID)
Dosage levels:	0 - 12.5 - 25 - 50% (w/v) in dimethylformamide (DMF)
Route:	Epidermal (topical) application on the dorsal ear lobe surface
GLP/QAU:	Signed documents available

Three groups each of four female mice were treated daily with Benzophenone-3 at concentrations of 12.5, 25 and 50% (w/v) in dimethylformamide (DMF) by topical application to the dorsum of each ear lobe (left and right) for three consecutive days. A control group of four mice was treated with the vehicle (DMF) only. Five days after the first topical application the mice were injected intravenously into a tail vein with radio-labelled thymidine (^{3}H-methyl thymidine). Approximately five hours after intravenous injection, the mice were sacrificed, the draining auricular lymph nodes excised and pooled per group. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes which were subsequently washed and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was determined by the incorporation of ^{3}H-methyl thymidine measured in a β-scintillation counter.

A test item is regarded as a sensitizer in the LLNA if the exposure to one or more test concentrations results in a 3-fold or greater increase in incorporation of ^{3}HTdR compared with concurrent controls, as indicated by the Stimulation Index (S.I.). The estimated concentration of test item required to produce a S.I. of 3 is referred to as the EC_3 value.

All treated animals survived the scheduled study period.
Stimulation Indices of 1.64, 1.33 and 1.61 were determined with the test item at concentrations of 12.5, 25 and 50% (w/v) in DMF.

The study authors conclude that the test item Benzophenone-3 was not a skin sensitizer under the described conditions.

Ref.: 13

3.3.4. Dermal / percutaneous absorption

3.3.4.1 *In vitro* dermal / percutaneous absorption - human skin

In a publication of 1999, Benzophenone-3 formed part of a battery of five UV filters for which standard operating procedures for their rapid analysis in various skin layers, were established. Benzophenone-3 was included at 4.9% in a cosmetic formulation (composition not stated) applied at 3 mg/cm² on fresh dermatomed (± 344 µm) human skin (6 samples from different donors) put on static diffusion cells. The 3 ml receptor fluid (pH 7.4) was maintained at 32°C and consisted of 1% bovine serum albumin, 0.9% NaCl, 0.02% KCl and 0.04% gentamycin in distilled water. The transepidermal water loss (TEWL) was recorded at each site with a Tewameter. After an exposure time of 16 hours, the skin was washed and dried with cotton swabs. The receptor fluid was collected and 16 strippings were carried out on the skin surface to determine the stratum corneum (SC) content and subsequently the epidermis was separated from the dermis. Analysis was preformed by isocratic RP-HPLC[2] with UV detection.

Benzophenone-3 quantification led to the following results:

Total amount applied	147 µg/cm² (3mg cream/cm², 4.9% Benzophenone-3)
Stratum corneum (SC)	8.5 ± 3.3 µg/cm²
Epidermis	0.3 ± 0.2 µg/cm²
Dermis	0.4 ± 0.1 µg/cm²
Receptor fluid	1.0 ± 0.4 µg/cm²
Washing solution	85.7% ± 4.5%
Recovery	93.4% ± 3.1%

The results indicate that the SC adsorbed the greatest proportion of the applied amount (5.8%), while about 0.5% was absorbed in the viable skin and 0.7% was analyzed in the receptor fluid.
According to the study authors the test can be considered as valid since the recovery was in the accepted range of above 90%.

They estimate the dermal absorption of Benzophenone-3 in respect to bioavailability after topical application to freshly dermatomed human skin for 16 hours as 1.7 µg/cm² (1.0 µg/cm² receptor fluid, 0.4 µg/cm³ dermis, 0.3 µg/cm² epidermis), corresponding to 1.16% of the applied dose.

Ref.: 54

Comment
The following shortcomings can be noted:
- The test concentration of 4.9% is lower than the maximum allowed level of 10%.
- The solubility of the Benzophenone-3 in the receptor fluid at 32°C is not stated. This is essential, since the compound's solubility in water is very low (0.0037g/l at 20°C).

[2] Reverse Phase - High Performance Liquid Chromatography

- The composition of the cosmetic formulation is unknown.
- Not all details on preservation and storage of skin are given.
- The purity of the test substance is not stated.
- Only one concentration of the test substance is used.
- 16 hours of contact is rather unusual. Normally, contact time is 24 hours.
- 6 samples from different donors is less than the requested amount.
- Only measurements after 16h are available; no intermediate sampling has been performed.

Several other studies were published dealing with *in vitro* skin penetration or certain aspects thereof using Benzophenone-3 (labelled or unlabelled) or Benzophenone-3 containing products. New or modified penetration models, different analytical methods as well as newly composed and/or different formulations were investigated and/or compared. The different working groups used human skin (full thickness or split thickness), pig skin (full thickness or dermatomed) or artificial membranes as test systems.
Each study has its own limitations since either no complete penetration but only penetration in certain skin compartments were investigated and reported (stratum corneum, epidermis, dermis), the application duration was changing (ranging from 30 minutes up to 10 hours at maximum) or several deficiencies in respect to methodology and/or reporting, when compared to guideline requirements exist.
Therefore only general statements can be derived from these studies, such as the fact that the dermal absorption of Benzophenone-3 appears to be low, that the major proportion is adsorbed by the stratum corneum and that certain formulations (o/w emulsion, w/o emulsion, gels, oils, creams) can influence the absorption rate in respect to time-course and amount of absorption.

Ref.: 10, 26, 27, 32, 54, 71, 72

3.3.4.2 *In vivo* dermal / percutaneous absorption - human

The published human studies (2002-2003) all concern the tape stripping methodology and they differ in duration of application, analytical methods, calculation of adsorption/absorption and in the composition of the formulations used. Therefore, no quantitative conclusion for the *in vivo* dermal absorption of Benzophone-3 is possible. Qualitatively it can be stated that, as was the case in the *in vitro* studies, the stratum corneum adsorbed the greatest fraction of the applied Benzophenone-3 and that only small amounts could be considered as absorbed and systemically bioavailable. In addition, the type of preparation/formulation had a clear influence on the extent of dermal absorption.

Ref.: 10, 27, 71

Comment
The following recent study on dermal absorption of Benzophenone-3 in volunteers has been added by the SCCP:

In 2006, after the submission of the dossier, Gonzalez et al. published another human study on the dermal absorption of Benzophenone-3 after repeated whole-body applications, with and without UV irradiation. 25 volunteers applied 2 mg/cm² of a sunscreen containing 4% of Benzophenone-3 to their whole body surface area, twice daily for 5 consecutive days. The amount of sunscreen per application varied between the participants and ranged from 26 g to 47 g. The volunteers were divided in two groups, of which one received UV-irradiation. During the 5 days of application, all urine was collected and analyzed for Benzophenone-3 concentration through high-performance liquid chromatography with UV detection.
The test results indicate that there was a large variation in the total amount of Benzophenone-3 excreted in the urine, even after compensation for the differences in body surface area. However, UV-irradiation did not affect the urinary secretion of the compound. The mean value of Benzophenone-3 found in the urine was 3.7% (1.2% - 8.7%). Other excretion routes were not investigated, thus this value may still be an underestimation of the total dermally absorbed percentage of Benzophenone-3. The volunteers excreted

Benzophenone-3 many days after the last application, which could be expected viewing the lipophilicity of the molecule.

Ref.: D

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated dose (28 days) oral / dermal / inhalation toxicity - rat/mouse

A. Subacute oral administration to rats and mice

A report dated 1953 describes a 27-day study in rats (10 animals per group) with dosages of 7.2, 75 and 789 mg/kg bw/day. Food consumption and body weight gains of the test groups were comparable to those of the control rats. There were no deaths and no significant gross pathologic changes in any of the animals which could be attributed to the administration of the test substance.

Ref.: 34

14-day oral toxicity in rat (1985-88)

Five F344/N rats per sex and group received Benzophenone-3 in concentrations of 0, 3,125, 6,250; 12,500; 25,000 and 50,000 ppm in the diet for 2 weeks. The dietary test substance preparations were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

303 mg/kg bw/day:	increased liver weights in males and females; increased kidney weights in males
576 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; increased kidney weights in males
1,132 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; increased kidney weights in males
2,238 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; increased kidney weights in males
3,868 mg/kg bw/day:	reduced feed consumption in males and females; reduced body weight gain in males; increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; increased kidney weights in males; focal dilatation of renal tubules in the cortex and/or medulla

Study authors' conclusion: NOAEL (14d-oral) = 295/311 mg/kg bw/day for the male/female rat.

Ref.: 30

14-day oral toxicity in mouse (1985-88)

Benzophenone-3 was administered in the diet to five B6C3F1 mice per sex and group at concentrations of 0, 3,125; 6,250; 12,500; 25,000 and 50,000 ppm for 2 weeks. The dietary test substance preparations were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals. At termination of treatment, all

animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

1,021 mg/kg bw/day:	increased liver weights in males and females
2,041 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes
4,430 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes
8,648 mg/kg bw/day:	increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; decreased kidney weight in males
20,796 mg/kg bw/day:	reduced body weight gain; increased liver weights in males and females associated with the presence of cytoplasmic vacuolisation of hepatocytes; decreased kidney weight in males

Study authors' conclusion: NOAEL (14d-oral) = 992/1050 mg/kg bw/day for the male/female mouse.

Ref.: 30

B. Subacute dermal administration to rats and mice

14-day dermal toxicity in rat (1985-88)

Five F344/N rats per sex and group received Benzophenone-3 at dose levels of 0, 1.25, 2.5, 5.0, 10.0, 20.0 mg/rat in acetone or lotion as vehicle for 5 days per week for 2 weeks. A constant volume of 0.25 ml/rat was applied over a fixed standard area (10%) of the interscapular region. The area was clipped 24 hours prior to initial application and weekly thereafter. The preparations in acetone and the lotion were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

7.0 mg/kg bw/day:	no adverse effects noted
13.6 mg/kg bw/day:	no adverse effects noted
27.7 mg/kg bw/day:	slightly increased liver weights in females
54.9 mg/kg bw/day:	slightly increased liver weights in female
110 mg/kg bw/day:	slightly increased liver weights in females; slightly increased kidney weights

Study authors' conclusion: NOAEL (14d-dermal) = 100/140 mg/kg bw/day for the male/female rat.

Ref.: 30

28-day dermal toxicity in rat (1985-88)

A publication of 1995 describes how 6 male Sprague-Dawley rats per group received Benzophenone-3, formulated in petroleum jelly base, at dosage levels of 0 and 100 mg/kg bw/day, twice daily for 4 weeks. Clinical examinations covering clinical signs and body weight were performed in all animals. Blood samples for haematology and clinical chemistry were taken prior to the start of treatment and on day 16. At termination, the animals were sacrificed, organs were weighed (liver, kidney, testes) and liver, kidneys, testes and skin from treated and untreated area were collected and histopathologically

examined. In addition, on day 16 of treatment, blood samples for GSH determination were collected.
No animal died premature. There was no substance-related effect on body weight, relative organ weights, haematological and clinical-chemical parameters. Physical examination of the skin revealed no substance-related changes. Histopathological examination of livers, kidney and testes revealed no significant difference between control and treated animals and no abnormalities were observed in the skin from treated or untreated areas.

Ref.: 51

14-day dermal toxicity in mouse (1985-88)

Five B6C3F1mice per sex and group received Benzophenone-3 preparations at dose levels of 0, 0.5, 1.0, 2.0, 4.0, 8.0 mg/mouse in acetone or lotion as vehicle for 5 days per week for 2 weeks.
A constant volume of 0.1 ml was applied over a fixed standard area (10%) of the interscapular region. The area was clipped 24 hours prior to initial application and weekly thereafter. The preparations in acetone and the lotion were analyzed for stability. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (applied doses were converted to dosages):

24.8 mg/kg bw/day: none
48.4 mg/kg bw/day: none
100 mg/kg bw/day: none
196 mg/kg bw/day: increased liver weight
388 mg/kg bw/day: increased liver weight

Study authors' conclusion: NOAEL (14d-dermal) = 384/432 mg/kg bw/day for the male/female mouse.

Ref.: 30

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

A. Sub-chronic oral administration to rats and mice

90-day oral toxicity in rat (1) (1972)

Benzophenone-3 (source and batch not cited) was examined in 12 male and 12 female rats per sex and group at dosages of approximately 0, 20, 100, 500 and 1,000 mg/kg bw/day for 13 weeks. The animals were observed for clinical findings and body weight and food consumption was determined weekly. Blood samples for haematology were collected in the 6^{th} and 12^{th} week. In addition, at termination liver enzyme activities and clinical chemistry parameters were examined in six rats per sex and dosage group. All animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted:

No deaths occurred.
20 mg/kg bw/day: no adverse effects noted
100 mg/kg bw/day: no adverse effects noted
500 mg/kg bw/day: reduced body weights and body weight gains; reduced haemoglobin and leukocytosis (with an increase in lymphocytes and decrease in neutrophils) after 6 weeks in females; anaemia,

	lymphocytosis and reduced number of granulocytes after 12 weeks in females; decreased absolute weights of the thymus and heart in males and females; decreased relative weights of the pituitary gland, thymus, heart and adrenal glands in males and females; first stages of degenerative nephritis in kidneys of males and females
1,000 mg/kg bw/day:	reduced body weights and body weight gains; ruffled fur and rigid limbs (reversible within 4 weeks); reduced haemoglobin and leukocytosis (with an increase in lymphocytes and decrease in neutrophils) after 6 weeks in females; anaemia, lymphocytosis and reduced number of granulocytes after 12 weeks in females; decreased absolute weights of the thymus, heart, pituitary gland, lungs, spleen, adrenal glands, and gonads in males and females; decreased relative weights of the pituitary gland, thymus, heart and adrenal glands in males and females; decreased relative weights of the lungs and spleen in females; increased relative thyroid weight in females; degenerative nephritis in kidneys of males and females

Study authors' conclusion: NOEL (90d-oral) = 100 mg/kg bw/day for male and female rats.
Ref.: 44

90-day oral toxicity in rat (2) (1985-1988)

Ten F344/N rats per sex and group received Benzophenone-3 in concentrations of 0; 3,125; 6,250; 12,500; 25,000 and 50,000 ppm in the diet for 13 weeks. The dietary test substance preparations were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals at regular intervals. Samples for haematological and clinical-chemical examination and urinalysis were taken on days 3 and 15 and in week 12 of treatment. Sperm morphology/motility and vaginal cytology examinations were performed at dietary levels of 0; 3,125; 12,500 and 50,000 ppm. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

204 mg/kg bw/day:	coloured urine; increased liver weight
411 mg/kg bw/day:	coloured urine; increased liver weight; disturbed serum protein levels
828 mg/kg bw/day:	coloured urine; increased liver weight; disturbed serum protein levels
1,702 mg/kg bw/day:	decreased growth and body weight gain in males and females; coloured urine; enlarged kidneys with abnormal shape and granular surface; increased absolute and relative kidney weights in females; dilatation of renal tubules; increased liver weight; increased platelet counts; disturbed serum protein levels
3,458 mg/kg bw/day:	decreased growth and body weight gain in males and females; coloured urine; enlarged kidneys with abnormal shape and granular surface; increased absolute and relative kidney weights in males and females; dilatation of renal tubules; mild to moderate inflammation with fibrosis in the renal interstitium; increased liver weight; increased platelet counts; disturbed serum protein levels; reduced sperm motility in males, increase in estrous cycle of females

Study authors' conclusion: NOAEL (90d-oral) = 429/393 mg/kg bw/day for the male/female rat.

Ref.: 30

90-day oral toxicity in mouse (1985-88)

Ten B6C3F1 mice per sex and group received Benzophenone-3 in concentrations of 0, 3125, 6,250; 12,500; 25,000 and 50,000 ppm in the diet for 13 weeks. The dietary test substance preparations were analyzed for stability and proved to be stable in the diet for at least 3 weeks. Clinical examinations covering clinical signs, mortality, body weight and food consumption were regularly performed. Sperm morphology/motility and vaginal cytology examinations were performed at dietary levels of 0; 3,125; 12,500 and 50,000 ppm. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

554 mg/kg bw/day:	no adverse effects noted
1,246 mg/kg bw/day:	increased liver weight
2,860 mg/kg bw/day:	increased liver weight
6,780 mg/kg bw/day:	decreased body weight gain in males and females; increased liver weight; minimal cytoplasmic vacuolisation of hepatocytes
16,238 mg/kg bw/day:	decreased body weight gain in males and females; minimal renal lesions in males; increased liver weight; minimal cytoplasmic vacuolisation of hepatocytes; decreased sperm density and increased abnormal sperm in males; increased estrous cycle length in females

Study authors' conclusion: NOAEL (90d-oral) = 1068/1425 mg/kg bw/day for the male/female mouse.

Ref.: 30

B. Sub-chronic dermal administration to rats and mice

90-day dermal toxicity in rat (1985-88)

Ten F344/N rats per sex and groups received Benzophenone-3 preparations at dosage levels of 0, 12.5, 25, 50, 100, 200 mg/kg bw/day in acetone for 5 days per week over a period of 13 weeks. A constant volume of 0.25 ml/rat was applied over a fixed standard area (10%) of the interscapular region. The area was clipped 24 hours prior to initial application and weekly thereafter. The preparations in acetone were analyzed for stability. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals at regular intervals.
Samples for haematological and clinical-chemical examination and urinalysis were taken on days 3 and 15 and in week 12 of treatment. Sperm morphology/motility and vaginal cytology examinations were performed at dosage levels of 0, 12.5, 50 and 200 mg/kg bw/day. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

The following effects were noted (dietary levels were converted to dosages):

12.5 mg/kg bw/day:	decreased reticulocyte count
25 mg/kg bw/day:	non-dosage related increase in relative kidney weight in females; decreased reticulocyte count
50 mg/kg bw/day:	non-dosage related increase in relative kidney weight in females; decreased reticulocyte count; increased platelet count
100 mg/kg bw/day:	non-dosage related increase in relative kidney weight in females; decreased reticulocyte count; increased platelet count

200 mg/kg bw/day: non-dosage related increase in relative kidney weight in females; decreased reticulocyte count; increased platelet count; increased whole blood cell count produced by lymphocytosis

Study authors' conclusion: NOAEL (90d-dermal) = 200 mg/kg bw/day for male and female rats.

Ref.: 30

90-day dermal toxicity in mouse

Ten B6C3F1 mice per sex and group received Benzophenone-3 preparations at dosage levels of 0, 22.8, 45.5, 91, 182, 364 mg/kg bw/day in acetone for 5 days per week over a period of 13 weeks. A constant volume of 0.1 ml was applied over a fixed standard area (10%) of the interscapular region. The area was clipped 24 hours prior to initial application and weekly thereafter. The preparations in acetone were analyzed for stability. Clinical examinations covering clinical signs, mortality, body weight and food consumption were performed in all animals at regular intervals. Sperm morphology/motility and vaginal cytology examinations were performed at dosage levels of 0, 22.8, 91, 364 mg/kg bw/day. At termination of treatment, all animals were sacrificed and macroscopically examined, organs were weighed and comprehensive histopathology was performed.

At all dosage levels, a mild increase in relative kidney weight in males, together with a decrease in epididymal sperm density, was noted. No other abnormalities were observed.

Study authors' conclusion: NOAEL (90d-dermal) = 364 mg/kg bw/day for male and female mice.

Ref.: 30

3.3.5.3 Chronic (> 12 months) toxicity

No data.

3.3.5.4 Overall conclusion of the submission authors on repeated dose toxicity

The toxicity of Benzophenone-3 after repeated application was comprehensively examined in subacute up to subchronic studies in rats and mice using the oral and dermal application route.
The systemic toxicity after repeated oral application was low and effects could mainly be observed at dose levels which were in the range or clearly above the current internationally accepted limit dose level of 1000 mg/kg bw/day for repeated toxicity studies. Beside unspecific signs of systemic toxicity in the form of reduced food consumption and retarded body weight gain, the identified target organs were the kidney and liver, partly associated with changes in clinical chemistry at high dose levels. Very often the most susceptible parameter was the increase in liver weight. However, this effect without any histopathological correlate does not reflect an adverse effect per se but is considered as an adaptive metabolic response, which is known to be reversible.

At very high dose levels clearly >3000 mg/kg bw/day in rats and >13000 mg/kg bw/day in mice after subchronic oral treatment, an impairment of selective reproductive parameters was noted. However, these are finally assessed in the reproduction section within this dossier (section 3.3.8 Reproductive toxicity).
Repeated dermal application of up to 13 weeks in rats and mice did not lead to any reliable substance-related local or systemic findings up to the highest dose level investigated in each case.
Finally, the reliable No-Adverse-Effect-Level (NOAEL) for subchronic toxicity after oral treatment was 6250 ppm (429/393 mg/kg bw/day in males/females) in rats and 6250 ppm (1068/1425 mg/kg bw/day in males/females) in mice.

For subchronic dermal treatment in each case the highest dose level was the reliable No-Adverse-Effect-Level (NOAEL), namely 200 mg/kg bw/day in rats and 364 mg/kg bw/day in mice.

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity/Genotoxicity *in vitro*

A. *In vitro* bacterial mutation assay (Ames test)

A number of publications (1980-1992) describe the results of Benzophenone-3 studied in the Ames test. The level of detail provided in these publications ranges from a summary and a title page [36] to a more extensive description of the materials and methods [73].
Generally the test substance was tested for mutagenicity in the reverse mutation assay on bacteria both, with and without metabolic activation (S9 mix from the liver of Aroclor 1254-induced male Sprague-Dawley and male Syrian hamster or rat livers). The mixes were prepared immediately prior to use and contained 10% S9. The tested Salmonella typhimurium strains were combinations of TA97, TA98, TA100, TA1535, TA1537 and/or TA1538 and were exposed to the test substance at various concentrations of Benzophenone-3 with and without S9 mix. Positive controls were included to demonstrate the sensitivity and validity of the test system used.
Bacteriotoxicity was reported [73] to be observed at a varying degree at and above 333 µg/plate and at 1000 µg/plate in all tested strains (TA98, TA100, TA1535, TA1537). In that same publication, Salmonella Typhimurium TA97 was additionally tested and showed a weak mutagenic response using 30% of S9 hamster mix.
However, no effect was noted using 10% hamster or 10% and 30% rat S9 mix compared to the respective controls, the increase in the numbers of revertants was less than 2-fold compared to the solvent control and there was no dose-response relationship. Benzophenone-3 showed to be negative for the induction of revertants in all other strains at the tested concentration range between 3 and 333 µg/plate. All other studies (not including TA97 in their testing battery) showed Benzophenone-3 to be negative in the Ames test.
Considering the fact that Benzophenone-3 did not induce gene mutations by base pair changes or frame shifts in the genome of the Salmonella typhimurium strains used in the presence and absence of S9-mix, the compound is considered to be non-mutagenic in the Ames test. The single weak positive response in one strain with a very high concentration of one specific S9 mix, is considered irrelevant by the authors of the submission.

Ref.: 8, 30, 36, 73

B. *In vitro* chromosome aberration test in Chinese Hamster Ovary cells

A US National Toxicology program report (1992) briefly describes the results of cytogenetic tests with Chinese hamster ovary cells, in which Benzophenone-3-induced sister-chromatid exchanges (effective dose range 5-50µg/ml) and chromosomal aberrations (20-45µg/ml) in the presence of Aroclor 1254-induced male Sprague Dawley rat liver S9.
One trial delivered a questionable result, while another trial appeared to be clearly negative.

Ref.: 30

Note
The cited reference for the chromosome aberration test (i.e. the NTP report) does not provide the level of detail displayed in the submission summary. Probably these details were extracted out of additional test descriptions / references not included in the submission.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

A. *In vivo* micronucleus test (Follow-up of the 90-day oral study in the mouse)

A US National Toxicology program report (1992) mentions that peripheral blood smears from the mice used in the 90-day studies as described under 3.3.5.2.A, were analyzed for the frequency of micronucleated normochromatic erythrocytes. No increase was noted in either male or female mice treated with up to 16,238 mg/kg bw/day of Benzophenone-3 administered orally.

Ref.: 30

B. *In vivo* rat bone marrow chromosome aberration test

A publication of 1995 describes how Benzophenone-3 was examined for its cytogenic potential *in vivo* in male and female Sprague-Dawley rats after a single or repeated (5 consecutive days) oral application by gavage. The test substance was dissolved in corn oil and was applied either once in dose levels of 0; 500; 1,670 and 5,000 mg/kg bw/day or for the repeated application at dose levels of 0 and 5000 mg/kg bw/day. Cclophosphamide was used as positive control substance and was orally administered as a single bolus or on five consecutive days at a dose level of 20 mg/kg bw/day. Since previously performed cell cycle kinetic studies investigating bromodeoxyuridine (BrdU) incorporation demonstrated that Benzophenone-3 did not affect the average generation time after single treatment of 5,000 mg/kg bw/day or after daily application for 5 days, bone marrow collection times were set at 8 and 12 hours after single application and at 12 hours after repeated administration.
Bone marrow smears were prepared, stained by fluorescence-plus – Giemsa and a total of 50 metaphase spreads from each animal were scored for chromosomal aberrations. In addition, the mitotic index was determined and the percentages of polyploid and endoreduplicated cells were analyzed.

No substance-related mortality occurred. The single treatment or the treatment for 5 consecutive days did not disturb the cell cycle and had no effect on the average generation time. No increase in the frequency of chromosomal aberrations was observed in bone marrow cells of male and female rats. Sex differences were not noted. The positive control substance caused chromosomal aberrations confirming the sensitivity of the test system.

The authors conclude that, since neither single nor repeated treatment up to 5,000 mg/kg bw/day in male and female Sprague-Dawley rats caused chromosomal aberrations, Benzophenone-3 was shown to be exhibit no clastogenic potential *in vivo*.

In that same publication, Benzophenone-3 is also reported to be investigated in the Drosophila somatic mutation and recombination test (SMART). Larvae from mated multiple wing hair females with heterocygotous flare males were fed a diet containing the test substance at concentrations of 0; 3,000 and 3,500 ppm for 72 hours. None of the fed larvae produced flies with significantly more single or multiple wing spots than negative controls. In contrast, positive control larvae produced flies with significantly more single or multiple wing spots than negative controls and confirmed the sensitivity of the test system. Finally, it was shown that Benzophenone-3 did not induce mutations, chromosome damage or genetic recombination in Drosophila using the SMART procedure.

Ref.: 55

3.3.6.3 Overall conclusion of the submission authors on mutagenicity/genotoxicity

Benzophenone-3 was tested in bacterial and mammalian test systems *in vitro*. No genotoxic/mutagenic potential was noted in three bacterial gene mutation assays in *Salmonella typhimurium* strains in the presence or absence of metabolic activation. The reported effect in a single strain after metabolic activation with an unusual high proportion was finally considered as irrelevant due to reporting and assessment deficiencies.

In mammalian cells systems, Benzophenone-3 showed no clastogenic potential and no ability to induce SCEs in the absence of metabolic activation. With metabolic activation a slight and/or non-concentration related increase in structural aberrations and the SCE rate were reported.

In vivo Benzophenone-3 was shown to be negative in the mouse micronucleus test after dietary administration for 13 weeks and in a chromosome aberration test in rats. Thus, the questionable effect observed *in vitro* was shown to posses no relevance for the *in vivo* situation.

Furthermore, Benzophenone-3 did not induce mutations, chromosome damage or genetic recombination in Drosophila using the SMART procedure.

3.3.7. Carcinogenicity

No data.

3.3.8 . Reproductive toxicity

3.3.8.1 Reproduction toxicity screening tests - rat/mouse

A. Oral reproduction toxicity screening tests

Reproductive toxicity pre-screening at the end of the 90-day oral toxicity in rat

Ten F344/N rats per sex and group received Benzophenone-3 in concentrations of 0; 3,125; 12,500 and 50,000 ppm in the diet for 13 weeks (stability of test substance in diet proven to be at least 3 weeks). At termination of the study, some specific examinations were performed to screen the potential reproductive toxicity of the substance. Observations in the male animals consisted of testicular, epididymal and caudal weights, sperm motility and morphology and sperm number per caudal tissue weight. Observations in the females included vaginal cytology examinations and estrual cyclicity.

The following effects were noted (dietary levels were converted to dosages):

204 mg/kg bw/day:	no deviations among the reproductive parameters studied
828 mg/kg bw/day:	no deviations among the reproductive parameters studied
3,458 mg/kg bw/day:	males: decreased right caudal, testicular and epididymal weights; decreased sperm number per caudal tissue females : prolonged cycle length.

Study authors' conclusion: NOAEL (reproduction) = 828 mg/kg bw/day for male and female rats.

Ref.: 24, 30

Reproductive toxicity pre-screening at the end of the 90-day oral toxicity in mouse

Ten B6C3F1 mice per sex and group received Benzophenone-3 in concentrations of 0; 3,125; 12,500 and 50,000 ppm in the diet for 13 weeks (stability of test substance in diet proven to be at least 3 weeks). At termination of the study, some specific examinations were performed to screen the potential reproductive toxicity of the substance. Observations in the male animals consisted of testicular, epididymal and caudal weights, sperm motility and morphology and sperm number per caudal tissue weight. Observations in the females included vaginal cytology examinations and estrual cyclicity.

The following effects were noted (dietary levels were converted to dosages):

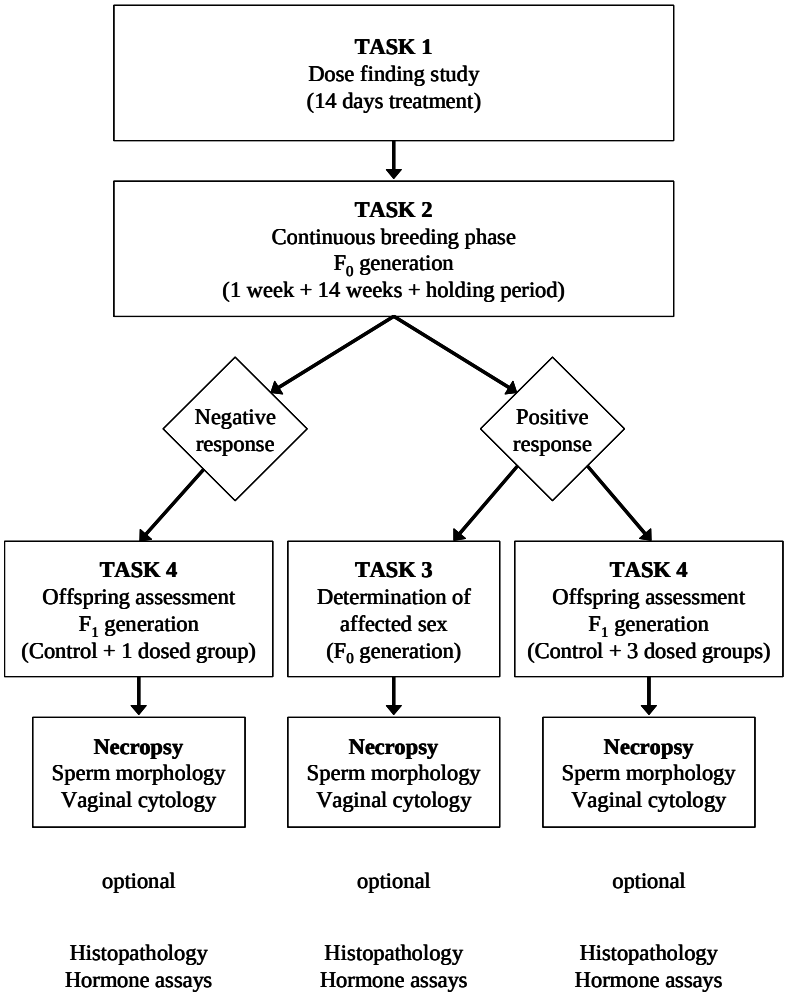
554 mg/kg bw/day:	no deviations among the reproductive parameters studied
2,860 mg/kg bw/day:	no deviations among the reproductive parameters studied
16,238 mg/kg bw/day:	decreased sperm number per caudal tissue and increased incidence of abnormal sperm in the males

Study authors' conclusion: NOAEL (reproduction) = 2,860 mg/kg bw/day for male and female mice.

Ref.: 24, 30

Reproductive Assessment by Continuous Breeding (RACB) in the mouse (1991)

The "Continuous Breeding Protocol" is a test design used by the US National Toxicology Program. It consists of four related tasks (see figure below), which are not all necessarily performed for every compound tested.



In the performed study, **Task 1** consisted of administering Benzophenone-3 in the diets[3] of groups of 8 CD-1 mice per sex at dosages of 0; 1,000; 2,100; 4,700; 10,200 and 15,700 mg/kg bw/day for 14 days. During the continuous breeding phase (**Task 2**), groups of 20 mice per sex were fed diets containing the test substance at dosage levels of about 1,850; 3,950 and 9,050 mg/kg bw/day, while the controls consisted of 40 animals per sex fed with unsupplemented diet. Feeding was started 1 week prior to mating and was continued for another 21 weeks. During this period the mice were cohabitated for 14 weeks (study week 2 to 17). Five litters were delivered during the whole study. The endpoints comprised clinical signs, body weight and food consumption, reproductive and fertility parameters and developmental endpoints in the progeny. The 1 week cross-over mating trial to determine the affected sex (**Task 3**) was not performed in this study, since no noted impairment of fertility occurred.

Finally, in **Task 4**, the development of the offspring was investigated using the last litter from Task 2. This progeny was reared, weaned and kept until mating (at 74 ± 10 days). At sexual maturity, a male and female from different litters were mated. The examinations in this phase were identical to the parents with the addition for checking the presence of a copulatory plug. The body weights were measured and estrous cyclicity was monitored by vaginal lavage 12 days prior to necropsy. In the males, epididymal sperm motility, sperm morphology and sperm count were investigated. At termination, necropsy was performed, organs were weighed and preserved for histopathology (especially reproductive organs).

The following effects were noted:

1,850 mg/kg bw/day:	5 dams died unexpectedly during the Task 2 20-day period
3,950 mg/kg bw/day:	reduced number of pups / litter; reduced dam weights; 4 dams died unexpectedly during the Task 2 20-day period
9,050mg/kg bw/day:	reduced number of pups / litter; reduced dam weights; 9 dams died unexpectedly during the Task 2 20-day period

Study authors' conclusion: NOAEL (fertility) = 8600/9500 mg/kg bw/day for male/female mice.

Ref.: 9, 31

B. Dermal reproduction toxicity screening tests

A short communication published in 1993 describes the assessment of the reproductive toxic potential of Benzophenone-3 in male B6C3F1 mice after a 13-week repeated dermal application of dosages of 0, 20, 100 and 400 mg/kg bw/day. Reproductive organ weights, cauda epididymal sperm concentration, proportion of motile and abnormal sperm and testicular spermatid concentration were determined and testicular histology was evaluated. Since neither any effect on body weight gain nor any abnormalities in the measured reproductive parameters were noted, the study authors conclude that topically applied Benzophenone-3 has no reproductive toxic potential in male B6C3F1 mice at dosages up to 400 mg/kg bw/day.

Study authors' conclusion: NOAEL (reproduction) = 400 mg/kg bw/day for male and female mice.

Ref.: 21

Finally, the results of the examinations of sperm morphology/motility and vaginal cytology in the dermal sub-chronic toxicity studies as described under 3.3.5.2.B, revealed a decrease in epididymal sperm density in the male mice. However, this finding is considered incidental

[3] Stability of Benzophenone-3 in the diet : at least 3 weeks

since there was no other effect on reproductive organs in the study, neither in the other selective reproductive parameters, weight of the reproductive organs nor histopathological examination. Therefore the NOAEL for the investigated reproductive effects in male and female rats and mice was in each case considered the highest dose level applied, more specifically 200 mg/kg bw/day for the rat and 364 mg/kg bw/day for the mouse.

Ref.: 30

3.3.8.2. Teratogenicity - rat - oral

Date of study:	01-16 November 2004
Guideline/method:	Annex V to Dir. 67/548/EEC, Method B.31; OECD Guideline 414
Species/strain:	Crl : WI(Han) Wistar rat
Group size:	25 mated females per dosage group
Test substance:	Benzophenone-3
Batch:	101
Purity:	99.8% (GC-FID)
Dosage levels:	0, 40, 200 and 1000 mg/kg bw/day
Route:	Oral (by gavage)
GLP/QAU:	Signed documents available

Benzophenone-3 was administered as a suspension in corn oil to 25 time-mated female rats per group by gavage at dosages of 40; 200 and 1,000 mg/kg bw/day on day 6 through day 19 post coitum (p.c.). A standard dose volume of 5 ml/kg body weight was used for each group. The control group, consisting of 25 females, was dosed with the vehicle (corn oil) in parallel. The oily test substance preparations were analyzed for stability prior to the study and for correct concentrations and homogeneity.
Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health of the animals was checked on a daily basis.
On day 20 post coitum all females were sacrificed and assessed by gross pathology (including weight determinations of the unopened uterus and the placenta). For each dam, corpora lutea were counted and number and distribution of implantation sites (differentiated as resorptions, live and dead foetuses) were determined. The foetuses were removed from the uterus, sexed, weighed and further investigated for external findings. Thereafter, nearly one half of the foetuses of each litter were examined for soft tissue findings and the remaining foetuses for skeletal (inclusive cartilage) findings.

The following effects were noted:

40 mg/kg bw/day:	no test-substance related effects on dams, gestational parameters or foetuses
200 mg/kg bw/day:	transient salivation in 3/25 rats immediately after dosing; no test-substance related effects on gestational parameters or foetuses
1,000 mg/kg bw/day:	transient salivation immediately after dosing; stained/reddish coloured urine; reduced food consumption and body weight (gain); slightly increased rates of foetuses/litter with skeletal variations (incomplete ossification of different skull bones and cervical arch, supernumerary 14th ribs(s)) and as a consequence increased rates of total variations; no test-substance related effects on gestational parameters

The performing laboratory concludes that Benzophenone-3 did not display any teratogenic effect and that the NOAEL for maternal and prenatal developmental toxicity is 200 mg/kg bw/day.

Ref.: 11

3.3.8.3 Overall conclusion of the submission authors on reproductive toxicity

Benzophenone-3 was shown to have no effect on fertility when tested up to very high dose levels within a RACB (Reproductive Assessment by Continuous Breeding) study in mice. The NOAEL for fertility was the highest investigated dosage level of 8600/9500 mg/kg bw/day in male/female mice. Within this study the reproductive performance was affected in the form of a slightly lower number of live pups at birth. Signs of developmental toxicity consisted of impaired body weight/body weight gain of the pups. However, in any case these effects were only noted at a dosage level with overt parental toxicity. Consequently, the NOAEL for systemic, reproductive and developmental toxicity was 1800/1900 mg/kg bw/day in males/females.

Repeated **oral** application of Benzophenone-3 for 13 weeks to rats and mice caused only slight effects in selective parameters accompanied with overt systemic toxicity at the highest investigated dosage levels of 3656/3261 mg/kg bw/day in male/female rats and 13937/18539 mg/kg bw/day in male/female mice). In rats, the epididymal sperm count was reduced and a decreased absolute cauda, epididymal and testis weight as a consequence of the reduced body weight was noted. In female rats, an increase in the length of the oestrous cycle was noted. In mice, a decrease in the epididymal sperm count and an increase the incidence of abnormal sperm was recorded, while female mice (as in rats) revealed an increase in the length of the oestrous cycle. However, in rats and in mice, the oestrous cyclicity was not affected. In any case, the next lower investigated dose level was a clear NOAEL for the investigated reproductive parameters.

After subchronic **dermal** application for 13 weeks no clear or reliable effect on selective reproduction parameter was noted and therefore, the NOAEL was in each case the highest dose level applied, namely 200 mg/kg bw/d in rats and 364 mg/kg bw/d in mice. Although in mice a decrease in the epididymal sperm count was reported at all investigated dose levels, a relation to treatment is considered as very unlikely since in the oral study the administration of dose levels up to 16-fold higher had no effect. Moreover, repeated dermal application of Benzophenone-3 to male mice of another strain for the same period led to no signs of reproductive toxicity up to the slightly higher dose level of 400 mg/kg bw/d. Thus, the reported effect on the sperm count was finally considered as incidental.

A recent prenatal developmental toxicity study performed according to valid test guideline and under GLP conditions with characterized test material resulted in marginal effect on few components of the skeleton was at the high dose level in association with overt maternal toxicity, the achieved NOAEL was 200 mg/kg bw/d for maternal and prenatal developmental toxicity.

3.3.9. Toxicokinetics

In a study published in 1986, the disposition of Benzophenone-3 in rats dosed orally, intravenously and topically, has been investigated.
[^{14}C]Benzophenone-3 was administered orally at dosages of 3, 28, 293 and 2570 mg/kg, dermally at approximate dosages of 0.2, 0.6, 0.8 and 3.2 mg/kg and intravenously at a dosage of 4.6 mg/kg. The dermal dosage of 0.6 mg/kg involved the use of a sunscreen lotion as vehicle, while the other dermal dosage levels concerned alcoholic solutions of the compound.
Through all routes and dosages, Benzophenone-3 appeared to be well-absorbed and urinary secretion clearly showed to be the major route of elimination, followed by the faecal route. Only trace amounts appeared to be measured in tissues after 72 hours.

The absorption rates did not differ between topical application of the compound in ethanol compared to the sunscreen lotion, indicating that there is no major vehicle effect on the dermal absorption of Benzophenone-3.

Five metabolites were identified and mainly consisted of glucuronide and sulfate conjugates.
Ref.: 23, 30

A US research group published three papers in 1993-94 describing the metabolism and disposition of Benzophenone-3 when administered orally in rats and mice and dermally in the rat at a uniform single dosage of 100 mg/kg bw.
The same metabolites are detected in all cases: 2,4-Dihydroxybenzone (DHB), 2,2'-dihydroxy-4-methoxybenzone (DHMB) and 2,3,4-trihydroxybenzophenone (THB). They have been identified in their free and conjugated (glucuronidated or sulfonated) forms.
However, some species-differences became very clear. More specifically, in dermally or orally exposed rats, the primary elimination route clearly is urine, followed by feces, whereas in mice, the excretion was divided between urine and fecal routes. In addition, the elimination from the plasma occurs via a biphasic model in the rat, while the mouse obeys a one-compartment elimination model. There also appears to be a higher level of accumulation in the rat liver and kidney compared to the mouse.
The authors attribute the major disparities in metabolism to the species-differences in cytochrome P-450 isoenzyme expression patterns that exist between rats and mice.
In both species and for both exposure routes tested, Benzophenone-3 was rapidly absorbed, metabolized and distributed.

Ref.: 49, 50, 52

The submission contains a paper of 2004 investigating the human dermal absorption and effect on reproductive hormone levels of 3 UV-filters after a whole body topical application of a highly concentrated sunscreen. In this study, 32 volunteers were treated with 2 mg/cm² of a basic cream formulation on a daily basis for 4 days during the first week, followed by the same treatment regime with a sunscreen containing 30% of UV-filters in total (10% 4-Methylbenzylidene Camphor, 10% Benzophenone-3 and 10% Ethylhexyl Methoxycinnamate) during the second week. Blood was collected at several time intervals on the first day of treatment and subsequently on a daily basis.

All three compounds were detected in their parent forms both in plasma (Benzophenone-3 up to 300 ng/ml) and urine, showing that there is a substantial skin penetration, dermal uptake and urinary excretion in humans. The systemic concentrations achieved did not affect any hormone level measured (testosterone, follicle-stimulating hormone, sex hormone binding globulin, luteinizing hormone, estradiol) under the conditions of the test.
Ref.: 38

The results of a study of 2002 investigating the urinary content of Benzophenone-3 after topical application to human volunteers can be considered as an indication for low bioavailability. A commercial available sunscreen containing 4% Benzophenone-3 was topically applied in an amount of 40 g to the average body area of 2.0 m² of each of 11 volunteers and urine samples were collected subsequently during 48 hours. Although the urine is known as the major excretion route for absorbed and bioavailable material, only 0.4% (corresponding a median of 9.8 mg/volunteer) of the applied Benzophenone-3 dose was recovered in the urine within the 48 hours sampling period.

Ref.: 33

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity/photoirritation and photosensitisation

A. *In vitro* phototoxicity

In the ECVAM[4] validation study of the 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT), Benzophenone-3 was included in the battery of UV-filters employed to check the accuracy and repeatability of the proposed *in vitro* assay.
The 3T3 NRU PT test makes use of Balb/c 3T3 mouse fibroblasts which are incubated for 60 minutes with several concentrations (usually eight) of the test compound. Thereafter the cells are exposed to a sun simulator for 50 minutes. After 24 hours the neutral red uptake (NRU) is measured and the respective EC_{50} values are defined as the concentrations of the test material which cause 50% reduction of NRU compared to the untreated control cultures. Subsequently the Photo Irritation Factor (PIF) is calculated. The PIF is defined as the factor generated by comparing two equally effective cytotoxic concentrations (EC_{50}) of the test chemical obtained in the absence (-UV) and in the presence (+UV) of a noncytotoxic irradiation with UVA/vis light [PIF = EC_{50}(-UV) / EC_{50}(+UV)]. If the PIF is $\geq$ 5, the substance is considered phototoxic. If a chemical is only cytotoxic +UVA and not when tested -UVA, the Mean Photo Effect (MPE) is calculated by a special computer software. In case the MEP, a measure which is based on comparison of the complete concentration response curves, is $\geq$ 0.1, the substance is considered phototoxic.
The validation study states that for Benzophenone-3 no animal data are available for comparison. Out of the 11 laboratories who have tested the compound through the *in vitro* 3T3 NRU PT protocol, the results for Benzophenone-3 were below the respective cut-off criteria for phototoxicity with the exception of one single PIF value of >2.7 and one MPE value of 0.195 obtained by one single lab. This single event was considered as incidental.

Ref.: 61

Benzophenone-3 was tested in another 3T3 NRU phototoxicity test in Balb/c fibroblasts and was found to be not phototoxic.
This conclusion was reinforced by the results of two separate photohemolysis and haemoglobin photo-oxidation tests, respectively performed with human erythrocytes and sheep red blood cells. Both assays showed that Benzophenone-3 had no phototoxic potential.

Ref.: 48, 53

Some other *in vitro* methods including the use of Saccharomyces Cerevisiae or Escherichia Coli plasmids as test organisms, showed Benzophenone-3 to be non-phototoxic.

Ref.: 40, 47

<u>*In vivo* phototoxicity in guinea pigs</u>

In a publication of 1999, the authors refer to a study published in 1974 in which the phototoxic potential of Benzophenone-3 was examined *in vivo* in guinea pigs. Five albino Hartley guinea pigs received a 0.05 ml aliquot of a 10% solution of Benzophenone-3 in petrolatum on two dorsal sites of the previously shaven and depilated skin. One application site was covered with aluminium foil. After 30 minutes, the uncovered site was irradiated with ultraviolet light (UV-A) for 60 minutes. Skin reactions were assessed 24 and 48 hours after irradiation, according to standard scoring systems (grade 0-4). No skin reactions were seen in any animal at any application site at any time point.

Ref.: 48

[4] European Centre for the Validation of Alternative Methods

B. *In vitro* Photosensitisation

In vitro Photosensitisation (mechanistic *in vitro* test)

Viewing the fact that the 3T3 NRU PT study does not enable to make the distinction between phototoxicity and photosensitisation, Benzophenone-3 was tested for its human serum albumin photobinding and histidine photo-oxidation potential in a newly proposed mechanistic *in vitro* test for the discrimination of the photo-allergic and photo-irritant potential of various test substances. Benzophenone-3 revealed no phototoxic and no photo-allergenic potential in this test.

Ref.: 45

In vivo Photosensitisation in rabbits

Although a short description of the test is provided in the submission summary, the appropriate reference [25] is missing in the dossier.
The test was claimed to be negative.

Ref.: 25

Phototoxicity and photosensitisation : human data

The dossier contains very brief descriptions of three unpublished reports (1978-1980) on the clinical safety assessment of sunscreens or other cosmetic products containing Benzophenone-3 up to 3.5% (full compositions are unknown). These older reports were supplied and assessed by the cosmetic ingredient review panel and published in the "Final Report on the Safety Assessment of Benzophenones-1, -3, -4, 5, -9, and -11 (J. American Coll. Toxicol., 2, 1983)". The conclusion of these reports is that Benzophenone-3 appears to have no phototoxic or photoallergenic potential when used as a cosmetic ingredient.

Ref.: 16, 29, 69

Finally, a number of publications of dermatological departments are available in which the authors display the result of a photopatch test with a number of UV filters including Benzophenone-3, in a population of patients with a history of photosensitisation. The results of these studies are summarized in the following table:

Survey period	# of patients	Country code	UV-filters tested	pos.* reactions	Remarks	Ref
1985-1990	187	USA	Benzophenone-3	9	- most reactions to sunscreens observed in last 3 years - trend towards increasing allergic response to Benzophenone-3 over time	22
			PABA**	1		
			Pentyl dimethyl PABA	2		
			Octyl Dimethyl PABA	5		
			Butyl Methoxy-Dibenzoylmethane	0		
1990-1993	108	IT	Benzophenone-3	4	The authors conclude that photocontact dermatitis caused by Benzophenone-3 is becoming more frequent confirming its increasing diffusion.	70
			PABA	1		
			Octyl Dimethyl PABA	0		
			Butyl Methoxy-Dibenzoylmethane	0		
			Isopopropyl Dibenzoylmethane	2		
			Ethylhexyl-*p*-methoxycinnamate	0		
			Isoamyl-methoxycinnamate	0		
			4-MBC	0		

Survey period	# of patients	Country code	UV-filters tested	pos.* reactions	Remarks	Ref
1982-1992	283	DK	Benzophenone-3	35	As in previous studies, Benzophenone-3 is the major cause for photocontact dermatitis in tested patients and photocontact dermatitis is much more frequent than contact dermatitis (practically not observed).	67
			PABA	3		
			Octyl Dimethyl PABA	14		
			Butyl Methoxy-Dibenzoylmethane	0		
			Isopopropyl Dibenzoylmethane	5		
			Ethylhexyl-*p*-methoxycinnamate	3		
			Isoamyl-*p*-methoxycinnamate	0		
			4-MBC	0		
1981-1996	402	DE	Benzophenone-3	9	Most of the photoallergic reactions appear to occur in the category of UVA absorbers. The authors advise to put in place a registry for adverse reporting of sunscreen agents in general.	57
			PABA	2		
			Octyl Dimethyl PABA	2		
			Butyl Methoxy-Dibenzoylmethane	13		
			Isopopropyl Dibenzoylmethane	32		
			Ethylhexyl-*p*-methoxycinnamate	4		
			Isoamyl-*p*-methoxycinnamate	10		
			4-MBC	5		
1990-1996	355	SV	Benzophenone-3	15	Photocontact reactions by far outnumbered contact reactions.	7
			PABA	2		
			Butyl Methoxy-Dibenzoylmethane	6		
			Isopopropyl Dibenzoylmethane	8		
			Ethylhexyl-*p*-methoxycinnamate	3		
			4-MBC	0		
1990-1994	370	FR	Benzophenone-3	21	Most of the positive cases to Benzophenone-3 were diagnosed before 1993. In France, the UV-filter is not being used in sunscreens any more; only in other cosmetics, such as daily moisturisers.	39
			PABA	1		
			Octyl Dimethyl PABA	1		
			Butyl Methoxy-Dibenzoylmethane	4		
			Isopopropyl Dibenzoylmethane	7		
			Ethylhexyl-*p*-methoxycinnamate	2		
			Isoamyl-*p*-methoxycinnamate	0		
			4-MBC	1		
2002	1	USA	Benzophenone-3	1	One specific case where both immediate and delayed hypersensitivity against Benzophenone-3 occurred.	43
1991-1997	1261	Central EU	Benzophenone-3	8		62
			PABA	3		
			Octyl Dimethyl PABA	0		
			Butyl Methoxy-Dibenzoylmethane	2		
			Isopopropyl Dibenzoylmethane	7		
			Ethylhexyl-*p*-methoxycinnamate	2		
			Isoamyl-*p*-methoxycinnamate	5		
			4-MBC	1		

* Positive reactions are restricted to photocontact allergy. Direct contact allergy is not taken into account in these figures.
** PABA = Para-amino Benzoic Acid

Ref.: 22, 70, 67, 57, 7, 39, 43, 62

Conclusion of the submission authors

- *Phototoxicity/photoirritation in vitro and in vivo*

In vitro Benzophenone-3 was comprehensively examined for its phototoxic potential within the frame work of the EU/COLIPA validation process. In respect to the weight of evidence Benzophenone-3 was proven to be not phototoxic in the 3T3 NRU assay, in the red blood cells assay or in human primary keratinocytes in the presence or absence of artificial sunlight. The failure to induce photohemolysis or photohaemoglobin oxidation was also confirmed independently in a further published red blood cell phototoxicity test.
In vivo there exists also no indication for a phototoxic potential in guinea pigs or mice. However, as the animals number in the guinea pig study was low and in mice, not primarily the phototoxic effect but the protective effect of broad-spectrum sunscreens was investigated, these investigations serve only for information.

- *Photosensitization in vivo*

The study investigating this endpoint is only available as secondary citation and the results should therefore be treated with caution. However, no photosensitization was reported after topical treatment of albino rabbits with a sunscreen containing 6% Benzophenone-3.
Finally, Benzophenone-3 can be regarded to be of no concern for photo-induced toxicity for humans.

Comment
The following publications were not included in the submission and have been added by the SCCP:

Survey period	# of patients	Country code	UV-filters tested	pos.* reactions	Remarks	Ref
1983-1998	2175	UK	Benzophenone-3	14	The authors conclude that, despite the large increase in the use of UV-filters over the last years, the development of photo-allergic reactions remains rare. Photopatch test series should be regularly reviewed and updated, but meanwhile, there is no evidence that photo-allergic reactions represent a common clinical problem.	A
			Benzophenone-10	10		
			PABA	5		
			Octyl Dimethyl PABA	5		
			Amyl Dimethyl PABA	2		
			Butyl Methoxy-Dibenzoylmethane	4		
			Isopopropyl Dibenzoylmethane	6		
			Isoamyl-methoxycinnamate	2		
			Ethyl-methoxycinnamate	2		
			Ethylhexyl-methoxycinnamate	2		
2000-2002	1155	UK IRL NL	Benzophenone-3	21	The publication discusses the methodology of photopatch testing in general and does not provide an assessment of the potential photo-allergy of the compounds tested.	B
			Benzophenone-4	6		
			PABA	1		
			Octyl Dimethyl PABA	2		
			Butyl Methoxy-Dibenzoylmethane	17		
			Isoamyl-methoxycinnamate	11		
			Ethylhexyl-methoxycinnamate	6		
			4-MBC	3		
2001-2003	82	Colombia	Benzophenone-3	22		C
			Octyl Dimethyl PABA	1		
			4-MBC	1		

Survey period	# of patients	Country code	UV-filters tested	pos.* reactions	Remarks	Ref
			Ethylhexyl-methoxycinnamate	8		

* Positive reactions are restricted to photocontact allergy. Direct contact allergy is not taken into account in these figures.

3.3.10.2 Photomutagenicity / photoclastogenicity

A. *In vitro* photomutagenicity : bacterial mutation assay

Date of study: October - December 2004
Guideline/method: Annex V to Dir. 67/548/EEC, Method B.13/14; OECD Guideline 471
Test system: Salmonella Typhimurium strains TA1537, TA98, TA100 and TA102
Test substance: Benzophenone-3
Batch: 101
Purity: 99.8% (GC-FID)
Doses tested: 3; 10; 33; 100; 333; 1,000; 2,500 and 5,000 µg/plate
GLP/QAU: Signed documents available

Benzophenone-3 was investigated for its potential 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 TA 1537, TA 98, TA 100, and TA 102. The test was performed in two independent experiments. Each concentration, including the controls, was tested in triplicate and concentrations of 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate in experiment I of 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate in experiment II were investigated. The test substance was dissolved in DMSO. Prior to the main experiments, the induction of toxicity and mutagenicity was investigated in a pre-experiment with all strains.

Toxic effects evident as a reduction in the number of revertants, were observed in the preexperiment (without irradiation) and in both main experiments in nearly all strains used. The plates incubated with the test item showed normal background growth up to 5000 µg/plate in all strains used. No substantial increase in revertant colony numbers of any of the four tester strains was observed following treatment with Benzophenone-3 under irradiation with artificial sunlight 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.
The sensitivity and validity of the test system used was demonstrated by the expected induction of a significantly increased number of revertants with the appropriate positive controls.

The study authors conclude that Benzophenone-3 did not induce gene mutations by base pair changes or frameshifts in the genome of the bacterial strains used and was therefore shown to be non-photomutagenic in this Salmonella typhimurium photomutagenicity test.
Ref.: 14

B. *In vitro* photomutagenicity : chromosome aberration test

Date of study: November 2004 - February 2005
Guideline/method: Annex V to Dir. 67/548/EEC, Method B.10; OECD Guideline 473
Test system: V79 Chinese Hamster lung cell lines
Test substance: Benzophenone-3
Batch: 101
Purity: 99.8% (GC-FID)
Doses tested: 3.1; 6.3; 12.5; 25.0; 50.0 and 75.0 µg/ml
GLP/QAU: Signed documents available

Benzophenone-3 was investigated for its potential to induce structural chromosomal aberrations in V79 Chinese Hamster cells in the absence and the presence of artificial sunlight in two independent experiments. A xenon burner with an additional special filter glass, emitting visible light and UVA/UVB light (ratio: about 30:1) > 290 nm was used as light source. The cultures were pre-incubated with the test item for 30 min. After pre-incubation, the cultures were exposed to 225 mJ/cm² UVA (Exp. I and II) or 375 mJ/cm² UVA (Exp. II). Three hours after start of treatment, the cultures were washed. Corresponding cultures with the test item were kept in the dark for the 3 hrs exposure period. The chromosomes were prepared 18 hrs (Exp. I) and 28 hrs (Exp. II) after start of treatment. Two parallel cultures were investigated and at least 100 metaphase plates were scored for structural chromosome aberrations in each culture, except for the positive controls, where only 50 metaphase plates were scored.
The highest applied concentration in the pre-test on toxicity (2,230 µg/ml, approx. 10 mM) was chosen with regard to the molecular weight of the test item in line with requirements of the current OECD Guideline 473. Dose selection for the cytogenetic experiments was performed considering the toxicity data and the occurrence of test item precipitation.

In the absence and the presence of irradiation, toxic effects were observed in both experiments as indicated by clearly reduced mitotic indices or cell numbers of below 50 % of control. However, partly concentrations showing clear cytotoxicity could not be scored for cytogenetic damage. In Experiment I and II, in the absence and the presence of irradiation, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment. The statistically significant differences to the solvent control were observed occasionally in this study but were considered as biologically irrelevant due to the lack of dose-dependency and the values were clearly within the respective historical control data ranges.
No relevant increase in the frequencies of polyploid metaphases was found after treatment with the test item when compared to the controls and the range of the historical control data.
The sensitivity of the system was demonstrated since the positive controls induced statistically significant increases in cells showing structural chromosome aberrations.

The study authors conclude that Benzophenone-3 did not induce structural chromosome aberrations in the absence or presence of artificial sunlight as determined by the chromosomal aberration test in V79 Chinese Hamster cells and was thus shown to be non-clastogenic in this chromosomal aberration photomutagenicity test when tested up to cytotoxic concentrations.

Ref.: 12

C. Additional information

A publication of 2001 describes the plasmid-relaxation assay as a rapid screening system for the detection of photogenotoxic chemicals. Benzophenone-3 showed to be negative in this assay.

Ref.: 47

D. Conclusion of the submission authors with regard to photomutagenicity/photoclasto-genicity

Benzophenone-3 was tested in bacterial and mammalian test systems according to valid testing guidelines and under GLP conditions with the characterized test material. No photogenotoxic/photomutagenic potential was noted in the bacterial gene mutation assays in *Salmonella typhimurium* strains and no photoclastogenic potential was recorded in the chromosome aberration test in Chinese hamster V79 cells, both with and without irradiation.
In addition, a published screening test revealed no indication that Benzophenone-3 may cause DNA damage with or without irradiation.

3.3.11 Human data

A human patch test (1965) showed that Benzophenone-3 was non-irritating to human skin after 24 hours of patching [59].

In a modified Draize Shelanski Repeat Insult Patch Test performed in 1979, approximately 300 mg of the test material *UV-9*[5] was applied to patch sites to the backs or volar forearms of 100 subjects at a concentration of 25% in petrolatum for ten alternate day 24 hour periods under occlusion. After a seven day rest period, challenge patches with 25% of *UV-9* in petrolatum were applied in the same manner to fresh sites on the backs or volar forearms of all subjects for 24 hours. There were no instances of irritation or sensitisation from the material in this test (scores remained 0 at all times) [41].

In 1978, a Human Repeat Insult Patch Test was performed with 2 sunscreen products called *Sun Tan Lotion* and *Protective Face Cream*[6]. 24 hour occlusive patches with about 200 mg of test substance were applied on the skin of 56 subjects, 10 times with a resting period of 24 hours in between. Ten to fourteen days after the last patch, the challenge patch was applied. None of the products was considered capable of inducing significant irritation or sensitisation [29].

Ref.: 59, 41

The submission summary contains a description of more than 10 human volunteer studies, not performed with Benzophenone-3 as such, but with representative products (mostly sunscreens) with a varying concentration of Benzophenone-3 (from 5% up to 10%). The main purpose of these studies is described to be the investigation of the safe usage of these products under enhanced and comprehensive use conditions. No irritation, allergic reaction, photo-irritation or photo-allergy related to the use of the tested sunscreens was noted. The full references of these studies are stated to be "available upon request".

Ref.: 76, 77, 78, 79, 80, 81, 82, 83, 85, 86, 87, 88, 89

3.3.12 Special investigations

Estrogenic potential

The submission authors state that this endpoint was not considered within this dossier since there is a very intensive evaluation of the SCCNFP (Opinion on the Evaluation of Potentially Estrogenic Effects of UV-filters adopted during the 17th plenary meeting of 12 June 2001) available. The final conclusion was that based on the actual scientific knowledge, the SCCNFP is of the opinion that the organic UV-filters used in cosmetic sunscreen products, allowed in the EU market today, have no estrogenic effects that could potentially affect human health.

Ref.: 56

Moreover, a recent study investigated whether 10% of Benzophenone-3 in a sunscreen formulation and 10% of other UV filters were absorbed and influenced endogenous reproductive hormone levels in humans after topical application. In this blinded study 32 healthy volunteers (15 young males and 17 postmenopausal females) received whole-body topical application of 2 mg per cm² of basic cream formulation without (week 1) and with (week 2) the sunscreens at 10% (wt/wt) daily. Benzophenone-3 was absorbed and maximum plasma concentrations were 200 ng/ml for females and 300 ng/ml for males. In

[5] Exact identity not stated.
[6] Composition unknown for both products

the urine, approximately 60 ng/ml was detected in females and 140 ng/ml in males The exposure of the sunscreen containing 10% Benzophenone-3 caused no effect on either of the examined hormones (FSH, LH, SHBG, estradiol, inhibin B, testosterone). Minor variations observed were considered to reflect the known and normal biological variations.
Ref.: 38

3.3.13 Safety evaluation (including calculation of the MoS)

Not applicable.

3.3.14 Discussion

The safety of Benzophenone-3 for its usage in sunscreen products for over the counter (OTC) products was first peer reviewed by the US FDA in 1978 [16]. Based on the data available at that time the FDA expert panel classified Benzophenone-3 as safe and effective. Subsequently, published and unpublished information on Benzophenone-3 including other Benzophenones were reviewed by an expert panel and published as cosmetic ingredient review (CIR) in 1983 [17]. The expert panel concluded on the basis of all available data and clinical human experience that Benzophenone-3 is safe for topical application to human skin in the present practices of use and concentrations in cosmetics.

Benzophenone-3 is a widespread UV-filter for which over the years a large amount of data have been generated, many of them between 1970 and 1988. This is reflected in the identification and physicochemical data section. The majority of the data are statements out of the technical and material data sheets. Only a number of determinations (quantification through capillary gas chromatography and part of the stability studies) have been performed according to GLP, clearly mentioning the batch tested and accompanied by a full description of the method. All other parameters are not individually referenced. Two of the references with regard to the identification of the substance (74, 75), are only "available upon request". They should have been included in the submission.

The quality of the toxicological dossier suffers from the fact that studies are often outdated and/or only available as publications in journals, with the result that on several occasions batch number and purity of the test substance are not mentioned, compositions of tested formulations are unknown, etc. Nevertheless the submission summary provides a comprehensive and well-structured overview of the available test descriptions and publications.

The UV-filter displays a low acute toxicity profile with oral and dermal LD_{50}-values exceeding the classification limit of 2000 mg/kg.

Benzophenone-3 is not considered as being irritating to the skin and the eyes. The studies to support this statement are unfortunately outdated and not performed according to current guidelines and GLP, but the human data with the compound under in-use conditions do not provide any indication of skin and eye irritation due to Benzophenone-3. Therefore additional testing in this area does not appear to be necessary.

Benzophenone-3 has been extensively tested for its photoirritating potential *in vitro* during the validation of the 3T3 NRU PT test and was found negative in the majority of cases.

With regard to the sensitising potential of the compound, two animal tests are available : a guinea pig Magnusson Kligman Maximisation test of 1978 and a LLNA of 2005. Both indicate that Benzophenone-3 is non-sensitising.

About 14 additional references, mainly consisting of repeated insult patch tests with Benzophenone-3 containing test formulations, are stated to be "available upon request" and

are described in the submission's summary. However, they do not add additional arguments to the discussion.

In addition, the submission contains a number of reports of clinical trials with regard to the photoallergenic potential of UV-filters in general. In each of these, a number of clear positive reactions to Benzophenone-3 are described. In the current report, some extra references on this issue have been added by the SCCP to the ones included in the submission. Looking at the positive photoallergic reactions to Benzophenone-3, it must be emphasized that the study population in all tests consisted of patients with a suggested history of photocontact allergy. As a general rule, results of clinical trials should be followed up in order to detect potential trends towards an increasing incidence of (photo)allergic reactions to specific compounds.
In the case of Benzophenone-3, the presented publications clearly indicate that the UV-filter is a photoallergen.

As far as the dermal absorption of Benzophenone-3 is concerned, some diverging results have been obtained. An *in vitro* study of 1999 generates a dermal absorption value of 1.7 µg/cm² or 1.16% of the applied dose, but the test suffers several shortcomings (tested concentration too low, solubility in receptor fluid not stated, composition of the tested formulation unknown, skin preservation and storage details not given, purity of test substance not stated, only one concentration tested, unusual contact time, insufficient skin samples and no intermediate sampling). All other *in vitro* studies indicate "low dermal absorption", but do not allow any quantitative determination.

Looking at the available *in vivo* human data, it is clear that Benzophenone-3 is absorbed through the skin to a certain extent, but again quantification is impossible. In one human study, where a 4% Benzophenone 3 sunscreen was applied at 2 mg/cm² on the whole body surface, the absorption was considered to be as low as 0.4 %. In another study, in which a sunscreen containing 10% Benzophenone-3 together with 10% 4-MBC and 10% Ethylhexyl Methoxycinnamate, the absorption appears to be higher, but no exact values are stated and the combination of the three UV-filters in one sunscreen at such high concentrations might influence the result.

A more recent study added by the SCCP and not included in the submission, shows that a sunscreen containing 4% of Benzophenone-3, could lead to a mean urinary excretion of 3.7% (1.2%-8.7%) of Benzophenone-3. However, in this study, again the compound was combined with two other UV-filters, it was not tested at the maximum requested concentration of 10% and other routes of excretion have not been considered.
Therefore, no conclusion can be drawn with regard to the dermal absorption of Benzophenone-3.

After repeated oral administration of Benzophenone-3 in rats and mice, the most frequently encountered adverse effects consist of some unspecific signs of systemic toxicity in the form of reduced food consumption and retarded body weight gain, together with some effects on the identified target organs being the kidney and the liver. These effects were partly associated with changes in clinical chemistry. Very often the most susceptible parameter was the increase in liver weight. The latter, however, without any histopathological correlate, is not considered by the submission authors to reflect an adverse effect per se but should be considered as an adaptive metabolic response which is known to be reversible. Therefore, according to the submission, the oral NOAEL corresponds to 411 mg/kg bw/day.

With regard to the results of the dermal repeated dose studies, a dermal NOAEL of 200 mg/kg bw/day is put forward, on the assumption that deviations without dose-response relationship and without correlated histopathological findings (e.g. the decreased reticulocyte count, increased relative kidney weight, increased platelet count and whole blood cell count in the 90d dermal study in rat) should not be taken into account.

It should be noted that, taking the complete set of oral and dermal subacute and subchronic toxicity studies together, the choice of the dosages may raise some questions. In the oral studies, the dosages appear to be extremely high (up to 20,796 mg/kg bw/day) whereas the dosage levels in the dermal studies appear to be very low (down to 7 mg/kg bw/day). Even though the results indicate that Benzophenone-3 causes adverse effects at lower dosages through the dermal route compared to oral administration, the dermal dosages remain at the low side and this is also confirmed by the absence of clear toxicity signs at the highest levels tested (as requested in the official EC B.9/OECD 410 and EC B.28/OECD 411 testing guidelines).

A recently performed and well-described teratogenicity study in rat showed Benzophenone-3 to be non-teratogenic under the conditions of the test. Only at the highest dosage level, which also caused maternal toxicity, some skeletal aberrations were noted. The NOAEL-value for maternal and developmental toxicity was 200 mg/kg bw/day.

Instead of a 2-generation study, the submission contains some specific reproductive toxicity parameter measurements made at the end of the subchronic toxicity studies described earlier, together with the description of a reproduction screening assay according to the "Continuous Breeding Protocol". Out of these results, a NOAEL value of 400 mg/kg bw/day for reproductive toxicity, was extracted. Although the test is not commonly performed within the EU regulatory framework and although a number of animals in all dosage groups unexpectedly died, it does not seem to be acceptable from an ethical point of view, to request a new 2-generation study with Benzophenone-3.

Toxicokinetic studies indicate that Benzophenone-3 is readily biotransformed into its three major metabolites 2,4-Dihydroxybenzone (DHB), 2,2'-dihydroxy-4-methoxybenzone (DHMB) and 2,3,4-trihydroxybenzophenone (THB), which have been identified in their free and conjugated (glucuronidated or sulfonated) forms. Excretion in the rat primary occurs via the urine, while in the mouse the fecal route appears to be equally important.

As far as the (photo)mutagenic/(photo)genotoxic potential of Benzophenone-3 is concerned, the presented *in vitro* and *in vivo* assays indicate that the substance does not possess (photo)mutagenic of (photo) genotoxic properties. With regard to the studies mentioned in the US National Toxicology Program report, the full text references of the summarised tests should have been provided.

4. CONCLUSION

It is the opinion of the SCCP that insufficient data are presented to calculate the Margin of Safety of Benzophenone-3 under the proposed conditions of use.

The following additional information is required:

- A dermal absorption study with Benzophenone-3 under its in-use concentrations (up to 10%) according to OECD Guideline 428 combined with SCCP/0970/06.

These data are requested before end of March 2007.

5. MINORITY OPINION

Not applicable

6. REFERENCES

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