Scientific Committee on Consumer Safety

SCCS

OPINION on benzophenone - 4

(CAS No. 4065-45-6, EC No. 223-772-2)

The SCCS adopted this document
during plenary meeting on 27 March 2024

ACKNOWLEDGMENTS

SCCS members listed below are acknowledged for their valuable contribution to the finalisation of this Opinion.

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 15 December to 16 February 2024). Comments received during this period were considered by the SCCS. For this Opinion, main changes occurred in the following section: 3.3.2, 3.5 as well as related sections of the discussion part and responses to questions 1 and 2 in the conclusion part.

All Declarations of Working Group members are available on the following webpage:
Register of Commission expert groups and other similar entities (europa.eu)

1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of benzophenone-4, does the SCCS consider benzophenone-4 safe when used as UV-Filter in cosmetic products up to a maximum concentration of 5%?*

   On the basis of the safety assessment, and considering the concerns related to potential endocrine disrupting properties, the SCCS is of the opinion that benzophenone-4 is safe when used as UV filter up to a maximum concentration of 5% in sunscreen, face and hand cream, all leave-on and rinse-off products (total dermal aggregate), lipstick, sunscreen propellant spray and pump spray, when used separately or in combination (based on deterministic aggregated exposure).

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of benzophenone-4 in cosmetic products?*

   Any additional use of benzophenone-4, such as protectant for stabilising cosmetic formulations when exposed to light, should remain within the concentration mentioned above *(i.e*. 5%), including the use as UV-filter.

3. *Does the SCCS have any further scientific concerns with regard to the use of benzophenone-4 in cosmetic products?*

   The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of benzophenone-4 for the environment.

Keywords: SCCS, scientific opinion, benzophenone – 4, Regulation 1223/2009, (CAS No. 4065-45-6, EC No. 223-772-2)

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on benzophenone – 4 (CAS No. 4065-45-6, EC No. 223-772-2) used in cosmetics products. preliminary version of 14 December 2023, final version of 27 March 2024, SCCS/1660/23

About the Scientific Committees

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems, which may pose an actual or potential threat.

These Committees are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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Doi ND

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

SCCS - Opinions (europa.eu)

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background on substances with endocrine disrupting properties

On 7 November 2018, the Commission adopted the review[1] of Regulation (EC) No 1223/2009 on cosmetic products ('Cosmetics Regulation') regarding substances with endocrine disrupting (ED) properties. The review concluded that the Cosmetics Regulation provides the adequate tools to regulate the use of cosmetic substances that present a potential risk for human health, including when displaying ED properties.
The Cosmetics Regulation does not have explicit provisions on EDs. However, it provides a regulatory framework with a view to ensuring a high level of protection of human health. Environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 ('REACH Regulation').
In the review, the Commission commits to establishing a priority list of potential EDs not already covered by bans or restrictions in the Cosmetics Regulation for their subsequent safety assessment. A priority list of 28 potential EDs in cosmetics was consolidated in early 2019 based on input provided through a stakeholder consultation. The Commission carried out a public call for data in 2019[2] for 14 substances (Group A)[3] and a second call in 2021[4] for 10 substances (Group B)[5] in preparation of the safety assessment of these substances. Benzophenone-4 is one of the above-mentioned substances for which the call for data took place.

Background on Benzophenone-4

Benzophenone-4 (CAS No. 4065-45-6, EC No. 223-772-2) with the chemical name '2-Hydroxy-4-methoxybenzophenone-5-sulphonic acid' is currently regulated as a UV-filter in sunscreen products in a concentration up to 5 % (Annex VI/22). Benzophenone-4 absorbs UV light across a broad range of UV wavelengths and, therefore, protects the skin from damage by UVB and UVA light. In addition, in the European database for information on cosmetic substances and ingredients (CosIng) Benzophenone-4 is also reported with the functions of 'UV-stabiliser' and UV-absorber' protecting cosmetic formulations against sunlight.
Benzophenone-4 has been subject to a safety evaluation by SCCNFP in 1999[6], where the committee concluded that *'…2-Hydroxy-4-methoxybenzophenone-5-sulphonic acid can be used safely in cosmetic sunscreen formulations at a maximum concentration of 5%, expressed as acid'* and proposed *'…no further restrictions or conditions for its use in cosmetic products'.*
During the call for data, stakeholders submitted scientific evidence to demonstrate the safety of Benzophenone-4 as UV-filter in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on Benzophenone-4 in view of the information provided.

[1] https://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-739-F1-EN-MAIN-PART-1.PDF
[2] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic%20products_en
[3] Benzophenone-3, kojic acid, 4-methylbenzylidene camphor, propylparaben, triclosan, Homosalate, octocrylene, triclocarban, butylated hydroxytoluene (BHT), benzophenone, homosalate, benzyl salicylate, genistein and daidzein
[4] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic-products-0_en
[5] Butylparaben, Methylparaben, Ethylhexyl Methoxycinnamate (EHMC)/Octylmethoxycinnamate (OMC)/Octinoxate, Benzophenone-1 (BP-1), Benzophenone-2 (BP-2), Benzophenone-4 (BP-4), Benzophenone-5 (BP-5), BHA/Butylated hydroxyanisole/tert-butyl-4-hydroxyanisole, Triphenyl Phosphate and Salicylic Acid
[6] https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/sccp_out57_en.htm

Terms of reference

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of benzophenone-4, does the SCCS consider benzophenone-4 safe when used as UV-Filter in cosmetic products up to a maximum concentration of 5%?*

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of benzophenone-4 in cosmetic products?*

3. *Does the SCCS have any further scientific concerns with regard to the use of benzophenone-4 in cosmetic products?*

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Benzophenone-4 (INCI)

3.1.1.2 Chemical names

IUPAC: 5-benzoyl-4-hydroxy-2-methoxybenzenesulfonic acid

Other names and synonyms:
2-Hydroxy-4-methoxybenzophenone-5-sulphonic acid
5-Benzoyl-4-hydroxy-2-methoxy- 2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid
Benzenesulfonic acid
Sulisobenzone

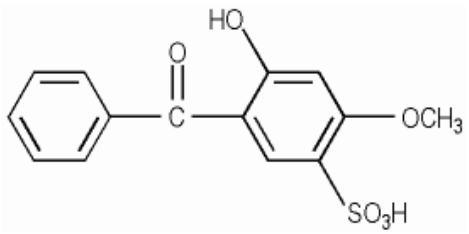
3.1.1.3 Trade names and abbreviations

HMBS, HMS, MS 40, Seesorb 101S, Spectra-sorb UV284, Sulisobenzona, Sungard, Syntase 230, Uvinuc ms 40, Uvinul MS 40, Uvinul ms 40, Ben-4 Eclipsogen BP4; Escalol 577

3.1.1.4 CAS / EC number

CAS: 4065-45-6
EC: 223-772-2

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{14}H_{12}0_6S$

3.1.2 Physical form

Pale, ivory-coloured powder

(CIR 2021)

3.1.3 Molecular weight

308.31 g/mol

3.1.4 Purity, composition and substance codes

Benzophenone-4 is prepared via sulfonation of benzophenone-3. The product is purified by precipitation from aqueous HCl, isolated by centrifugation, washed with acidic water and dried. The purity of the non-labelled compound is 98.5% (HPLC) and 97.9% (HPLC-UV), and the radiochemical purity of the radiolabelled compound is 99.5% (HPLC).

SCCS comment
Data on impurities per batch number for the batches used in this dossier must be provided.

3.1.5 Impurities / accompanying contaminants

The maximum recommended impurity concentration for lead and arsenic in benzophenone-4 are 18ppm and 1ppm respectively.

(CIR 2021)

SCCS comment
It was not stated which methodology was used; the impurities should be measured in the batches used here and these should be the same as the batches brought on the market.

3.1.6 Solubility

Water solubility: 313.7 g/l at 25°C (experimental value) (applicant dossier)
300.920 g/L at 25°C (ECHA)
2.5×10^5 mg/l at 25°C (O'Neil 2013)
1g dissolves in: 2ml methanol,
3.3ml ethanol,
4 ml water, and
100ml ethylacetate (O'Neil 2013)
Solubility in acetone, acetonitrile and toluene: < 1 mg/l at 26.7°C
Solubility in methanol and ethanol: between 200 – 1000 mg/l at 26.7°C
Solubility in dimethylformamide: between 100-200mg/l at 26.7°C

(applicant dossier, ECHA)

3.1.7 Partition coefficient (Log Pow)

Log P_{ow} = 0.313 at 22°C (experimental value, shake flask method) (applicant dossier)
Log P_{ow} = 0.515 ± 0.023 at 25°C (OECD Guideline 117, Reverse Phase HPLC) (ECHA)

3.1.8 Additional physical and chemical specifications

Melting point:	145°C at 101.3 kPa	(O'Neil 2013)
	120°C at 101.3 kPa	(applicant dossier)
	119.1-123.1°C at 97.24 kPa	(ECHA)
Boiling point:	491°C at 101.3 kPa	(applicant dossier)
	178.3°C at 97.17 kPa	(ECHA)
Relative density:	0.5 at 20°C	(applicant dossier)
Mean pour density:	0.2977 g/cm³ at 20°C and 97.34 kPa	(ECHA)
Tapped density:	0.3152 g/cm³ at 20°C and 97.34 kPa	(ECHA)
Vapour pressure:	0 Pa at 25°C	(applicant dossier)
	1.79E-009 Pa at 25°C	(ECHA)
pKa = 0.022 at 20°C		(applicant dossier)
pKa 1: -2.4 (sulfonic acid); pKa2=7.6 (hydroxyl)		(Chemspider)
pH (1 vol%):	1.92 at 26 °C	(ECHA)
UV absorption:	λ_{max} 288 nm	(SCCNFP/0085/98)
Particle size distribution (Granulometry): 150 micron to 75 micron		(ECHA)

When heated to decomposition, benzophenone-4 emits toxic vapours of sulfur oxides.
Storage conditions: Keep container tightly closed in a dry and well-ventilated place. Hygroscopic. Store under inert gas. Keep in a dry place.

(Sigma-Aldrich SDS)

Store below 40 °C (104 °F), preferably between 15 and 30°C (59 and 86 °F), unless otherwise specified by manufacturer.

(Thomson/Micromedex 2006)

3.1.9 Homogeneity and Stability

No data submitted.

SCCS comment
Data on the stability of the test substance under the experimental conditions of the reported studies and under conditions of use were not provided. The SCCS retrieved information in the public domain. According to ECHA registration dossier, benzophenone-4 was observed to be stable in organic solvent dichloromethane and no degradation products were formed after 24 hours as evident from the GC-MS chromatogram obtained at 0 hours and that obtained after 24 hours.

(ECHA)

SCCS comments on physicochemical specifications
The physicochemical data present in the submitted dossier were confusing. Further explanation was given at the request of the SCCS.
However, the stability of the test substance in the marketed product (and in the test solutions) was not reported.
A full report of the chemical characterisation of benzophenone-4 in terms of purity, identity and impurities in representative batches must be provided and the validity of the analytical methodologies used must be shown. Identity and concentration of any impurities that may be present must also be stated.

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

3.2.1.1 *In vitro* animal skin absorption data

In this *in vitro* study, hairless female Sprague Dawley rat skin was used to evaluate the absorption of radiolabelled test material. Franz diffusion cells were employed, where the stratum corneum was exposed to the air, and the dermal surface was in contact with 0.9% saline containing 15 mg/ml of bovine serum albumin. Three preparations of active ingredient were used, each at 5%, expressed as acid: an o/w emulsion; a hydroalcoholic gel; and an aqueous solution. The applied material contained a radioactive isotope (14-C active ingredient) with varying levels of radioactivity ranging from 0.1 to 0.5 µCi/mg. Abdominal skin from hairless rats was prepared by removing the fat with a scalpel. Applications of the formulations of active ingredient were made to the skin, and the fluid regularly sampled; each experiment lasted 24 hours. After cleaning the surface of the skin, the skin itself was mechanically separated into dermis and epidermis with a forceps, and these were separately extracted and counted. Each experiment was repeated 8 times. The contact surface area was 0.635cm^2. The percentage of the applied material found in the receptor fluid after 24 hours of contact was approximately 0.107 ± 0.008% for the emulsion, 0.096 ± 0.025% for the hydroalcoholic gel, and 0.143 ± 0.079% for the aqueous solution. In the epidermis, the recovered amounts were 3.66 ± 0.53% for the emulsion, 3.86 ± 0.28% for the hydroalcoholic gel, and 8.15 ± 1.0% for the aqueous solution. In the dermis, the amounts found were 2.04 ± 0.36% for the emulsion, 1.59 ± 0.45% for the hydroalcoholic gel, and 0.72 ± 0.11% for the aqueous solution. The majority of the applied material was recovered in the washing liquid, with 81.35 ± 5.12% for the emulsion, 78.92 ± 2.13% for the hydroalcoholic gel, and 84.48 ± 1.23% for the aqueous solution. Although some differences in permeation were found between the various formulations, these were not significant, and the mean values are as follows (in percentages of amounts applied, after 24 hours): epidermis, 5.22; dermis, 1.45; fluid, 0.12.

(Doucet O. & Wepierre J. 1991 cited in SCCNFP/0085/98)

An *in vitro* skin absorption study, equivalent to OECD Guideline 428, was performed on excised pig skin under occlusive conditions. A dose of 0.1 and 0.2 mg/cm² (i.e. 2 mg/cm² of a 5% and 10% formulation) was applied to the surface of the epidermal membranes for 24 hour duration. The exposed area was 3.14 cm² and the receptor chamber contained 7.0 ml of aqua bidest as the receptor medium, maintained at a temperature of 31.5-32.5°C. Various vehicles were tested, including DAE (40% N,N-Dimethylacetamide, 30 % Acetone, 30 %Ethanol); Gel (not further specified), Cream (not further specified and Aqua bidest (a double distilled water). The 10% benzophenone-4 dose was applied as a suspension in DAE, while the 5% benzophenone-4 dose in DAE was in solution. Both 5% and 10% benzophenone-4 were soluble in double distilled water. Samples for analysis were collected at various time points: 0, 0.5, 1, 3, 6, 16, and 24 hours after application. A volume of 1.0 ml was taken from the receptor chamber at each sampling, with the first 0.3 ml discarded and the remaining 0.7 ml used for analysis. The samples were stored at -20 °C until analysis, and the test substance concentration was determined through absorbance measurement. No data regarding the total recovery was provided. Penetration was the highest from the enhancing DAE vehicle (2.13% of the 10% dose and 11.29% from the 5% dose). The amount absorbed from the gel and water vehicles was not assessable as it was below the limit of quantification. In the cream vehicle, skin absorption through the pig epidermal membranes was 0.8% for 5% test solution and 0.4% for the 10% solution.

(unnamed 1997 study report in EU REACH record)

In a similar study equivalent to OECD Guideline 428 using pig skin epidermal membranes, 5% benzophenone-4 was tested under occlusive conditions in a 5% suspension in DAE (40% N,N-Dimethylacetamide, 30 % Acetone, 30 %Ethanol) and an oil:water emulsion (83g Aqua bidest (including 10M NaOH to pH neutralise + 12g paraffin oil DAB10 (10g)/Cremophor A25 (2g) mixture). The 5% test formulations were dosed at 2 mg/cm^2 leading to a dose of 0.1 mg/cm^2. The receptor medium temperature was kept at 31.5 - 32.0 °C throughout the experiment. The epidermal membranes were prepared according to the laboratory's standard operating procedure and stored at -20 °C until the study began. The concentration of the test substance was determined using HPLC. Sampling was performed at various time points (0, 30 mins, 1.0, 3.0, 6.0, 16.0 and 24.0 hours). A total of 1.0 ml of receptor medium was collected, with 0.3 ml discarded and 0.7 ml used for analysis. The study used different receptor media, including Aqua bidest, PBS, and a 0.9% aqueous NaCl solution. Occlusion was achieved using Parafilm. No data regarding the total recovery was provided. The results showed a percutaneous absorption rate of 5.9% for the test substance in the 5% suspension in DAE formulation (24 hours, mean, n=6) and 2.57% for the test substance in the 5% suspension in an oil/water emulsion formulation (24 hours, mean, n=11).

(unnamed 1998 study report in EU REACH record)

In another *in vitro* study following guidelines similar to OECD 428, 5% benzophenone-4 was tested in an oil-in-water emulsion using full-thickness and split-thickness pig skin. Pig skin was obtained from the flank of a domestic female pig from a slaughterhouse. The skin was cleaned, shaven, and stored at -20 °C until use. Full-thickness pig skin had a thickness of 2,500-3,500 µm; split-thickness membranes were prepared with an electric dermatome and had a thickness of 300-500 µm. Skin integrity was visually checked using a stereomicroscope. Two to four replicates were performed per tested compound. The test substance was applied at a dose of 2 mg/cm² to the skin samples. The results showed that the percutaneous absorption rate of the test substance was 4.03 (± 2.21%) in split-thickness pig skin and 2.63 (± 1.05%) in full-thickness pig skin after 16 hours of exposure at a dose of 2 mg/cm².

(Benech-Kieffer *et al*. 2000)

A study was performed to investigate the relative effect of vehicles on the skin penetration of benzophenone-4. Four different vehicles were assessed: petrolatum, oil:water and water:oil emulsions and hydroxyethyl cellulose (HEC) gel. For benzophenone-4, the highest skin penetration was seen when applied in a HEC gel, then in oil:water emulsion and the least penetration from petrolatum.

(Kurul and Hekimoglu 2001)

SCCS comment

The available *in vitro* skin penetration studies using rat and/or pig skin do not comply with the basic criteria for dermal absorption studies described in the 12th Revision of the SCCS Notes of Guidance. Therefore, these studies will not be used to estimate dermal absorption of benzophenone-4.

3.2.1.2 *In vivo* animal skin absorption data

Skin penetration of benzophenone-4 was tested on hairless male rats. Under anaesthesia, a grille was attached around the site of application to prevent licking or rubbing of the site. The rats were divided into four groups (each 6 rats) and treated with three different formulations: an emulsion, a hydroalcoholic gel, and an aqueous solution, all containing 14C labelled benzophenone-4 in the form of sodium salt at a concentration of 5%. 20 mg test item was applied to a 10 cm^2 area of the rat's dorsal skin for 6 hours, after which the site was cleaned and the animals placed in metabolism cages, as before.

As an emulsion, only a minimal amount of the ingredient was excreted in the urine. After 6 hours, only 0.14 ± 0.06% of the applied dose was excreted, and after 24 and 120 hours, the

percentages increased slightly to 0.19 ± 0.08% and 0.42 ± 0.12% respectively. The excretion rate was higher within the first 24 hours, and at equilibrium, approximately 0.06% of the dose was excreted every 24 hours. Washing the application site after 6 hours resulted in the recovery of 95.96 ± 3.66% of the dose, indicating low skin absorption. The bioavailability was calculated to be 0.5% for a surface area of 10 cm^2.

The hydroalcoholic gel shows a low excretion rate of 0.14 ± 0.06% of the applied dose after 6 hours of contact. After 24 hours, only 0.19 ± 0.08% is excreted in the urine, and this increases to 0.42 ± 0.12% after 120 hours of contact. The excretion is significantly higher in the first 24 hours compared to later time points, and at equilibrium, approximately 0.06% of the dose is excreted per 24 hours. Washing the application site after 6 hours of contact allows the recovery of 95.96 ± 3.66% of the dose. The bioavailability is estimated to be 0.5% for a surface area of 10 cm^2.

Similarly, the aqueous solution shows comparable excretion levels. After 6 hours of contact, urine excretion is 0.06 ± 0.03% of the dose, increasing to 0.12 ± 0.06% after 24 hours, and 0.37 ± 0.09% after 120 hours. At equilibrium, approximately 0.07% of the dose is excreted per 24 hours. Washing the application site after 6 hours of contact allows the recovery of 77.88 ± 4.45% of the dose. The bioavailability is calculated to be 0.44% for a surface area of 10 cm^2.
In conclusion, the compound showed poor absorption through the rat's skin *in vivo*, with only a small percentage (around 0.15%) being excreted after 24 hours of contact, regardless of the formulation used. The elimination continued throughout the study, with an approximate excretion rate of 0.06% per 24 hours. After 120 hours of contact, only 0.40% of the applied dose was absorbed. The bioavailability was similar for all three formulations, amounting to approximately 0.5% for a surface area of 10 cm^2.

(Doucet O. & Wepierre J. 1991 cited in SCCNFP/0085/98)

SCCS comment
As the *in vivo* study is old and was carried out in rats, it will not be used in the safety evaluation of benzophenone-4. The dermal absorption in rat skin is very low. It is to be expected that the dermal absorption in humans will also be limited.

3.2.1.3 *In vitro* human skin absorption data
Human abdominal skin, obtained at plastic surgery, was cut in a microtome to 300 µm thickness. The skin was held in a Franz cell. The stratum corneum was exposed to the air, and the dermal surface was in contact with 0.9% saline containing 15 mg/ml of bovine serum albumen. Three preparations of active ingredient were used, each at 5%, expressed as acid: an o/w emulsion; a hydroalcoholic gel; and an aqueous solution. The applied material contained 14-C active ingredient with varying levels of radioactivity ranging from 0.1 to 0.5 µCi/mg. The formulations of the active ingredient were applied to the skin, and samples of the fluid were regularly collected over a 24-hour period. After cleaning the skin's surface, the skin was separated into the dermis and epidermis, and each layer was individually extracted and measured for radioactivity. Each experiment was repeated eight times. The contact surface area was 0.635 cm^2. The percentage of the applied material found in the receptor fluid after 24 hours of contact was: 0.035 ± 0.013% for the emulsion, 0.0014 ± 0.003% for the hydroalcoholic gel, and 0.014 ± 0.002% for the aqueous solution. In the epidermis, the recovered amounts were 1.35 ± 0.23% for the emulsion, 1.29 ± 0.26% for the hydroalcoholic gel, and 1.72 ± 0.21% for the aqueous solution. In the dermis, the values were 2.24 ± 0.71% for the emulsion, 0.78% ± 0.20% for the gel, and 0.45 ± 0.09% for the aqueous solution. The washing procedure resulted in the recovery of 80.63 ± 4.48%, 77.76 ± 4.03%, and 89.92 ± 2.49% for the emulsion, hydroalcoholic gel, and aqueous solution, respectively. Although some differences in permeation were found between the various formulations, these were not significant, and the mean values are as follows (in percentages of amounts applied, after 24 hours): epidermis, 1.45; dermis, 1.15; fluid 0.02.

(Doucet O. & Wepierre J. 1991 cited in SCCNFP/0085/98)

In an *in vitro* study reported as equivalent to an OECD guideline 428 study, Potard *et al*. used static diffusion cells to evaluate the skin absorption of benzophenone-4. The substance was incorporated into a cream at a concentration of 6.9% and applied at a dose of 3 mg/cm^2 to human skin obtained from abdominal or breast surgery. The limit of detection (LOD) using a high-performance liquid chromatography method was determined to be 90 µg/l. After 16 hours, the amount of benzophenone-4 found in the receptor fluid was below the LOD; 1.9% was recovered from the stratum corneum and 93.6% removed from the skin surface. The total mass balance recovery from this experiment was 96 ± 4.2% with 0.048% epidermal recovery and 0.097% dermal recovery. Total skin absorption at 16 hours in this experiment was 0.145%.

(Potard *et al*. 1999)

In a separate study by the same group, the percutaneous absorption of benzophenone-4 in an oil:water emulsion was evaluated *in vitro* using fresh human skin of women (aged 20-65 years) who had undergone breast or abdominal surgery. Skin samples were prepared by removing subcutaneous fat after gentle cleansing with distilled water and a paper tissue. The skin thickness averaged around 350 ± 50 µm. The skin samples were either used immediately or stored at 4 °C for 24 hours. The skin (epidermal side up) was positioned on the lower part of the glass static diffusion cell, and 3 mg/cm^2 of the test formulation was applied. The receptor fluid used was phosphate-buffered saline at pH 7.4, comprising NaCl, KCl, phosphate buffer, bovine serum albumin, and gentamicin sulphate. The test temperature maintained an average skin membrane temperature of 32 °C. Transepidermal water loss of the skin was measured after a 30-minute stabilization period. A 3% caffeine emulsion served as the reference substance. At the end of the exposure time (30 minutes or 16 hours), any remaining test formulation on the skin surface was removed before stripping. This was accomplished by applying a washing solution of lauryl ether sulphate and water on the skin surface, which was then collected. The washing procedure was repeated three times, followed by drying and a final wash with water. Subsequently, the upper part of the cell was removed, and the skin was dried again. For mass balance purposes, all the washing solution, water aliquots, tips, cotton swabs, and the top of the cell were collected. To strip the stratum corneum, D-Squam® Tape was used to obtain sixteen strips. The tapes were applied and removed in different directions to ensure uniform stripping of the stratum corneum while minimizing damage to the dermo-epidermal junction. The tapes were stored at -18 °C for subsequent analysis. In the case of the 30-minute experiment, the first two tapes were separated, while the remaining tapes were pooled together for analysis. HPLC analysis, performed according to methods equivalent or similar to OECD Guideline 428, was conducted on all samples, including tapes, epidermis, dermis, receptor fluid and washing solution. The total recovery of the test substance was reported to be greater than 80%. The quantity of benzophenone-4 found in stratum corneum after 30 minutes (2.1 ± 1.3 µg/cm^2) was statistically significantly less than that found after 16 hours (4.0 ± 1.8 µg/cm^2). Benzophenone-4 was stated as zero or very low in receptor fluid.

(Potard *et al*. 2000)

In another OECD Test Guideline 428 equivalent study, 5% benzophenone-4 was tested in an oil-in-water emulsion using human skin obtained from surgical patients from the abdominal area. The skin was rinsed with de-ionized water and visually inspected for any alterations. Subcutaneous fat was removed, and the samples were stored at -20 °C until the day of the experiment. Skin membranes were prepared with an electric dermatome. Skin samples were fixed on a dissection board. Epidermal side up, and sections were cut at 300-500 µm. The dermatomed layer thus obtained included the epidermis and some dermal tissue. Three skin donors (one replicate per donor) were used. 2 mg/cm² of the test substance was applied to the surface of the skin samples. The results showed that the percutaneous absorption rate of the test substance was 6.18% (± 3.90%) in split-thickness human skin after 16 hours of exposure at a dose of 2 mg/cm².

(Benech-Kieffer *et al*. 2000)

SCCS comment
The above *in vitro* human skin absorption data are either from the open literature or studies claimed to be equivalent to OECD Test Guideline 428. However, they do not comply with the basic criteria for dermal absorption studies described in the 12th Revision of the SCCS Notes of Guidance (SCCS/1647/22). Therefore, these studies will not be used to estimate dermal penetration of benzophenone-4.

New *in vitro* human skin absorption study

Guideline:	OECD Test Guideline 428
Test system:	Fresh abdominal human skin collected from surgery and frozen at -20°C
Number of donors:	5 Caucasian female donors (between 36-61 years)
Skin preparation:	350-450 µm thick prepared with a dermatome
Membrane integrity:	Verified using TEWL measurement (all within acceptable range of 0.7 – 5 g/m^2/h)
Test substance:	Benzophenone-4
Test item:	A standard sunscreen formula base containing 5% benzophenone-4 long with various other ingredients such as Dicaprylyl ether, Isopropyl palmitate, Glyceryl stearate SE, Cetearyl alcohol, Dimethicone, Phenoxyethanol, Glycerin, Carbomer, Isopropyl palmitate, and Ammonium acryloyldimethyltaurate/vp copolymer.
Batch:	Non-labelled: 0000112277 (used for radiolabelled formulation) or 0000092220 (used for solubility testing) Radiolabelled: 12041CAL007-3 [phenyl-U-14C] benzophenone-4; SEL/12041/3, specific activity 180.35 µCi/g
Purity:	Non-labelled: 98.5% (HPLC-UV) Radiolabelled: 99.5 % radiochemical purity and 97.9% chemical purity (HPLC)
Dose applied:	2 mg/cm^2 of the test formulation corresponding to approximately 0.1 mg benzophenone-4/cm^2
Exposed area:	1 cm^2
Study period:	24 hours
Assay conditions:	32°C ± 1°C
Sampling:	At 30 minutes, 1, 2, 4, 8 and 24 hours
Receptor fluid:	0.01M phosphate buffered saline pH 7.4
Solubility in receptor fluid:	785 µg/ml
Mass balance analysis:	Provided
Tape stripping:	20 strips, pooled as: 1-2, 3-8, 9-14 and 15-20
Method of analysis:	Liquid scintillation counting
GLP:	In compliance
Period:	June 2021 – Nov 2021

Some deviation from the original study plan was noted. The homogeneity of the test items was checked on 2 mg instead of 5 mg to ensure the same amount as applied to the skin, and water was added to improve the mixing process. The coefficient of variation (CV) for the test item homogeneity was within acceptable limits. The density of the remaining samples was measured and stored at -20°C instead of +4°C, but it did not impact the results. The homogeneity of the test items was checked before and during application, and the CV was used as a measure of variability.

The results obtained are presented in Table 1.

Table 1: Individual and mean results (% recovery of applied dose) obtained following 14C-benzophenone-4 application to human skin after 24 hours.

Cell	1	2	3	4	5	6	7	8	9	10	11	12	Mean	SD
	Donor 1		Donor 2		Donor 3		Donor 4			Donor 5				
Test item applied mg/cm²	2.2	2	2.2	2	2.3	2.1	2	2.2	2.4	2.4	1.8	2.1	2.14	0.18
Total strips (%)	0.07	0.07	0.02	0.02	0.05	0.17	0.05	0.20	0.06	0.20	0.13	0.17	0.10	0.07
Skin excess (%)	100.57	107.07	101.04	100.48	112.04	93.09	74.74	112.37	64.98	96.86	118.77	79.44	96.62	16.20
Epidermis (%)	0.05	0.08	0.21	0.09	0.34	0.26	0.26	0.03	0.13	0.09	0.06	0.14	0.14	0.10
Dermis (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.14	0.02	0.07	0.02	0.06	0.02	0.04	0.04
Receptor Fluid at 24h (%)	BLOQ	BLOQ	BLOQ	BLOQ	0.01	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	0.01*	NC
Receptor Cell Rinse (%)	BLOQ	BLOQ	0.08	0.21	BLOQ	BLOQ	0.16	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	NC	NC
Absorbed (%)*	0.07	0.10	0.31	0.32	0.37	0.28	0.56	0.05	0.20	0.10	0.11	0.16	NC	NC
Total RECOVERY (%)	100.7	105.24	101.36	100.82	112.47	93.54	75.35	112.62	65.24	97.16	119.02	79.76	**96.9**	NC

NC – not calculated; *single observed value; Absorbed epidermis+dermis+receptor fluid with receptor cell rinse. BLOQ – Below the limit of quantification – 100dpm.

The total % recovery of the applied dose of benzophenone-4 at 24h is 96.9%. This is well within the SCCS acceptable range of 85-115% recovery in such experiments. In the interpretation of these data, it is important to note that observations of benzophenone-4 in receptor fluid and receptor cell rinse were so low that a statistical evaluation of these parameters is not meaningful. In almost all cases, levels were below the limit of quantitation (BLOQ where LOQ = 100dpm). However, the applicant is confident that the value of 96.9% is a realistic value in the experiment and would not be significantly influenced by any addition of quantitative data from the receptor fluid.
The total absorbed value in this study is calculated by adding the amounts recovered in epidermis + dermis + receptor fluid + receptor cell rinses. The range of dermal absorption values varied from 0.05% (0.05 µg-eq/cm^2) to 0.56% (0.56 µg-eq/cm^2), with most values leaning towards the lower end of the range. A single value >0.5% was due to a relatively high value for the receptor cell rinse, potentially caused by some low level contamination. Given the difficulty in performing a meaningful statistical evaluation on these data with so many observations below the limit of quantitation in the receptor fluid, a pragmatic conservative dermal absorption value of 0.5% was proposed for safety evaluation.

(Eurofins 2021)

SCCS comment
SCCS considers the *in vitro* skin penetration study from 2021 using human skin as the key study. The bioavailable amount of benzophenone-4 is considered to be 0.46% (mean value of 0.20% + 2 SD of 0.13%) of the applied dose. Two SD are necessary as a high variation in dermal absorption values was measured, one test concentration in one formulation was used and four individual samples did not meet the mass balance criterium of 85-115% at 24h. For another three conditions (30 min., 2h and 4h), the mean mass balance was also inferior to 85%.

3.2.1.4 *In vivo* human skin absorption data
In a study conducted by Couteau *et al*., benzophenone-4 was topically applied to the skin of 21 healthy women aged 22 to 34 years (mean age = 25 ± 3 years). A treated skin area of 4 cm^2 was covered with self-adhesive tapes to prevent any product loss from the sides. Benzophenone-4 was deposited on the skin by applying 25 µl of a 6 mg/ml solution in water and 25 mg of a 0,6% oil-in-water (O/W) emulsion (80% distilled water and cetearyl alcohol, ceteareth-12, paraffinum liquidum, BHT, propylene glycol, methyl paraben sodium and propyl paraben sodium). The stratum corneum was stripped at 1 and 7 hours after application using transparent adhesive tape by a series of six strippings. 10 ml of distilled water were added to the six strips, and each sample was shaken with a vortex mixer after which the concentration of benzophenone-4 was determined in these solutions using HPLC. The results showed that after 1 hour, 70% of benzophenone-4 remained in the stratum corneum when applied as aqueous solution. After 7 hours, 40% of the applied benzophenone-4 was still detected in the

stratum corneum, suggesting a gradual decrease in its presence over time. This indicates that dermal absorption of benzophenone-4 is very low as the vast majority of the applied dose benzophenone-4 had not penetrated through the stratum corneum into the deeper skin layers but remained in the upper layers of the stratum corneum. In case of the benzophenone-4 oil-in-water emulsion, a smaller remanence compared to the results obtained with the aqueous solution was observed. After 2 and 7 hours, only 45% and 20% of benzophenone-4 was detected in the stratum corneum, respectively. This indicates that incorporation of benzophenone-4 in an oil-in-water emulsion promotes its penetration.

(Couteau *et al*. 2001)

SCCS comment

The study of Couteau *et al.* (2001) comes from the open literature and does not provide enough details to be used in the safety evaluation.

SCCS overall conclusion on dermal absorption

All available *in vivo* and *in vitro* studies show that the dermal absorption of benzophenone-4 is limited.

SCCS considers the *in vitro* skin penetration study from 2021 using human skin as the key study. The bioavailable amount of benzophenone-4 is taken as 0.46% (mean value of 0.20% + 2 SD of 0.13%) of the applied dose. The use of 2 SD in determining the dermal absorption is in accordance with the SCCS Notes of Guidance, 12th revision (SCCS/1647/22). The reason is that a high variation occurred in the measured values, one test concentration in one formulation was used, four individual samples did not meet the mass balance criterium of 85-115% at 24h. For another three conditions (30 min., 2h and 4h) the mean mass balance was also inferior to 85%.

3.2.2 Other studies on toxicokinetics

3.2.2.1 Oral route ADME/kinetics

In a GLP compliant study, female Wistar rats (n=5 juvenile animals of 22 days old and n=2 adult animals of 88 days) were dosed twice daily to benzophenone-4 by oral gavage in water for 4 consecutive days. The test material was not radiolabelled. The nominal dose was 1000 mg/kg/day. The urine of each animal was collected for 24 hours from day 0 to 4 and analysed for the test item by HPLC. The urine samples of single juvenile animals per group (n=5) were pooled, respectively. Urinary samples of the adult animals (n=2 per group) were analysed individually. In juvenile animals, 9.4% of the orally applied dose was recovered from urine. For adult animals, the recovery in urine was higher, with approximately 23.3% and 16.6% of the administered dose, respectively.

(BASF AG 1998a)

SCCS comment

Benzophenone-4 has only been measured in the collected urine; no other compartments were examined in this study to assess the remaining body content of benzophenone-4 and the excretion via bile or faeces that would allow a full balancing to quantify the total benzophenone-4 fraction absorbed via the oral route. However, the results of this study demonstrate that benzophenone-4 is absorbed in juvenile and adult rats after oral administration and, at least in part, excreted via urine. The higher percentage of the administered dose found in the urine of adult rats compared to juvenile rats could be indicative of age-related differences in the metabolism or elimination of benzophenone-4.

The SCCS is of the opinion that the oral absorption as determined in this study cannot be used. Therefore, the default value of 50% will be used for the oral absorption in the MoS calculation.

3.2.2.2 Lung route ADME/kinetics

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3.2.2.3 Dermal route ADME/kinetics

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3.2.2.4 Intravenous route ADME/kinetics

Six hairless rats received intravenously 500 µl of a 0.065% solution of benzophenone-4 with 14C activity of 36 µCi/ml. They were then placed in individual metabolism cages and urine was collected over 120 hours. The results showed that a mean of 83.67% of the applied radioactivity was found in the urine; about 80% had been excreted in the first 24 hours.

(Doucet O. & Wepierre J. 1991 cited in SCCNFP/0085/98)

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

For many years, benzophenone-4 has been widely utilized in cosmetic products to safeguard the skin and hair from the harmful effects of the sun. It also serves as a product protectant by stabilizing cosmetic formulations when exposed to light.

Benzophenone-4 containing sun protection products have been shown to protect the skin from phototoxicity in individuals taking the medicines chlorpromazine, chlortetracycline and 3,3',4',5-tetrachlorosalicylanilide, all of which often produce photosensitivity in patients.

Cosmetic products containing benzophenone-4 can be applied to the skin or, incidentally, may come in contact with the eyes (eye makeup preparations). According to the applicant, concentrations up to 0.2% is safe.

Benzophenone-4 is also being used in aerosol hair spray with according to the applicant a maximum concentration of 0.015%, and in pump hair spray with maximum concentrations of 0.1%.

(CIR 2021)

3.3.2 Calculation of SED/LED

Dermal exposure:

An OECD Test Guideline 428 *in vitro* skin penetration study from 2021 using human skin showed that topical application of 2 mg/cm^2 radiolabelled benzophenone-4 at 5% in a standard sunscreen formula base resulted in a dermal delivery of 0.20 + 0.13% after a 24h exposure period. The SCCS decided to use 0.46% (mean + 2SD) from this skin penetration study for SED and MoS calculation.

Dermal exposure:

Benzophenone-4 at 5% in sunscreen lotion:

Description	Parameter	Value	Unit
Estimated daily amount applied	$E_{product}$	18	g/day
Concentration benzophenone-4	C	5	%
Dermal absorption	DAp	0.46	% of applied dose
Bodyweight	BW	60	kg
Systemic exposure dose	SED	0.069	mg/kg bw/day

Benzophenone-4 at 5% in leave-on face cream:

Description	Parameter	Value	Unit
Relative daily exposure	$E_{product}$/bw	24.14	mg/kg bw/day
Concentration benzophenone-4	C	5	%
Dermal absorption	DAp	0.46	% of applied dose
Systemic exposure dose	SED	0.006	mg/kg bw/day

Benzophenone-4 at 5% in hand cream:

Description	Parameter	Value	Unit
Relative daily exposure	$E_{product}$/bw	32.70	mg/kg bw/day
Concentration benzophenone-4	C	5	%
Dermal absorption	DAp	0.46	% of applied dose
Systemic exposure dose	SED	0.008	mg/kg bw/day

Dermal aggregate to benzophenone-4 at 5% in leave-on and rinse-off products:

Description	Parameter	Value	Unit
Estimated daily amount applied	$E_{product}$	17.4	g/day
Concentration benzophenone-4	C	5	%
Dermal absorption	DAp	0.46	% of applied dose
Bodyweight	BW	60	kg
Systemic exposure dose	SED	0.067	mg/kg bw/day

Oral exposure:

Benzophenone-4 at 5% in a lip stick:

Description	Parameter	Value	Unit
Relative daily exposure	$E_{product}$/bw	0.9	mg/kg bw/day
Concentration benzophenone-4	C	5	%
Dermal absorption	DAp	100	% of applied dose
Systemic exposure dose	SED	0.045	mg/kg bw/day

Inhalatory exposure:

Benzophenone-4 at 5% in sunscreen pump and propellant spray products:

Tier 1 Inhalation exposure – to pump and propellant spray products

A systemic exposure dose from the potential for inhalation (SEDinh) from spray products can be calculated by assuming instant release of the ingredient in a defined box (1-Box model) or by applying a 2-Box model based on principles in Rothe *et al.* (2011). In Section 3-3.5.4.1 and Appendix 11 of the 11th Notes of Guidance (SCCS/1628/21), a deterministic approach for 2-box modelling was presented for propellant and pump spray products (using sunscreen as an example product) according to the following generic equations and associated parameters:

$$SED_{inh} = (IA_1 + IA_2) \times G \times RF \times DA/BW$$

Where:

SED_{inh} = Systemic Exposure Dose from the inhalation route (mg/kg/day)

IA_1 = the potential amount inhaled during the first 2 min (in mg)

$$IA_1 (EA/V_1*BR*t_1)$$

IA_1 = the potential amount inhaled during the subsequent 10 min (in mg)

$$IA_2 (EA/V_2*BR*t_2)$$

EA = potential amount to be inhaled

$$EA = (A*C*P*AF)/100$$

A = Amount of product by application (mg/application) (user defined or as per SCCS 2021)*

C = Percentage concentration of ingredient in product (%) (user defined)*

P = Proportion of non-propellant in formulation (no units) (user defined)* or default 60% (propellant, 100% pump spray) RIVM 2006

AF = airborne fraction (no units); 1 (propellant spray); 0.2 (pump spray) (Bremmer et al 2006)

V_1 = First step: near-field, $1m^3$ (SCCS 2021)

V_2 = Second step: far-field, $10m^3$ (SCCS 2021)

BR = breathing rate, 13 L/min (SCCS 2021; US EPA 2011)**

t_1 = 2 minutes in near field (SCCS 2021)

t_2 = 10 minutes in far field (SCCS 2021)

G = default factor substance lung retention 0.75 (25% is exhaled) (Rothe et al 2011; SCCS 2021)

RF = respirable fraction: propellant & pump spray specific (user defined experimental value*)

DA = Daily frequency of application (user defined* or as per SCCS 2021)

BW = body weight = adult 60 kg (SCCS 2021)

*Product-dependent parameter value

** highest median among several adult age categories

The calculation of SEDinh for benzophenone-4 used as a UV filter in sunscreen lotion is presented in Table 2. This is expected to be the worst-case spray exposure scenario due to

the total daily amount of product exposure being 18 g/day, much higher than any other cosmetic product type.

Table 2: Tier 1 Systemic exposure dose (SED) after inhalation exposure to 5% benzophenone-4 in sunscreen propellant and pump spray products.

Description	Parameter	Propellant spray	Pump spray	Unit
Amount by application[1]	A	15000	9000	mg/application
Percentage of benzophenone-4 in non-propellant	C	5	5	% (as acid)
Proportion of non-propellant in formulation	P	0.6	1	-
Airborne fraction	AF	1	0.2	-
Potential amount to be inhaled	EA = (A*C*P*AF)/100	450	90	mg
First step: near-field, 1m^3	V_1	1000	1000	L
Breathing rate	BR	13	13	L/min
2 min in near field	t_1	2	2	min
Potential amount inhaled during t_1	$IA_1 = (EA/V_1*BR*t_1)$	11.7	2.34	mg
Second step: far-field 10m^3	V_2	10000	10000	L
Breathing rate	BR	13	13	L/min
10 min in far-field	t_2	10	10	min
Potential amount inhaled during t_2	$IA_2 = (EA/V_2*BR*t_2)$	5.85	1.17	mg
Substance availability fraction	G	0.75	0.75	-
Respirable fraction	RF	0.2	0.01	
Frequency of application	F	2	2	day^{-1}
Default body weight	BW	60	60	kg
SED_{inh}	$(IA_1+IA_2)*G*RF*F/BW$	**0.088**	**0.0009**	**mg/kg/day**

[1] Adjusted for the proportion of propellant to achieve and 'on body' amount of 9000mg

It is assumed that for both pump spray and propellant spray, the same amount of sunscreen needs to reach the skin to ensure the necessary level of sun protection. For a propellant spray, this means that the additional amount of propellant gas needs to be added to the default value of 9 g/application, resulting in 15 g/application. By applying a factor of 0.6 as typical for the proportion of non-propellant in the formulation, this results in an amount of 9 g/application on the skin (SCCS/1628/21).
The concentration of benzophenone-4 in the products is assumed to be the maximum allowable at 5%.

The airborne fraction (AF) of 1 for propellant sprays and 0.2 for pump sprays are assumptions taken for sunscreens from the SCCS 11th Notes of Guidance (SCCS/1628/21) example and as supported by Bremmer *et al*. (2006) and Delmaar and Bremmer (2009).

The near-field zone of the two-compartment model was assumed to have a volume V1 of 1 m^3 and the duration of staying in the near-field zone t1 as 2 min. For the far-field a volume V2 of 10 m^3 and a duration of 10 min (t2) was assumed.

The respirable fraction is product specific. The inhalation exposure to aerosol particles generated by either propellant or pump spray formats will be primarily driven by the particle size distribution. According to Rothe *et al.* (2011) exposure to larger particle sizes (>10 µm but < 70 µm) is limited to the upper respiratory tract and the tracheobronchial tree and correspond to the inhalable fraction (IF). The smaller particle sizes (<10 µm), which can reach the deep lung regions correspond to the respirable fraction (RF). The larger particles, especially those with particle sizes > 70 µm are likely to settle quickly and thereby not be available for inhalation exposure. Upon inhalation, deposition and absorption of large particles/droplets would occur in the upper airways depending on their physical chemical properties. Only particles of sizes < 10 µm may produce effects in the lower respiratory tract (Rothe *et al*. 2011). For typical spray products containing benzophenone-4, based on the total number of particles released during one spray activity, the respirable fraction (<10 µm) were measured to be less than 1% for pump spray and less than 10% for the aerosol sprays respectively (internal survey by Cosmetic Europe).

Summary on exposure to benzophenone-4 at 5%:

Product type	SED (mg/kg bw/day)
Dermal exposure:	
Sunscreen lotion	0.069
Face cream*	0.006
Hand cream*	0.008
Dermal aggregate for leave-on and rinse-off products	0.067
Oral exposure:	
Lip stick*	0.045
Inhalatory exposure:	
Sunscreen propellant spray*	0.088
Sunscreen pump spray	0.001
Overall aggregate* (deterministic)	*0.147*

3.4 TOXICOLOGICAL EVALUATION

3.4.1. Irritation and corrosivity

3.4.1.1 Skin irritation

In vitro data:

***In Vitro* Skin Corrosion: Reconstructed Human Epidermis (RhE)Test Method**

Guideline:	OECD Test Guideline 431 (July 2016)
Test system:	EpiDerm™ 200 model (0.6 cm^2)
Number of replicates:	Two tissues per condition
Test substance:	Benzophenone-4
Test substance No.:	02/0296-4
Test item:	Neat
Batch:	Y7115C182

Purity:	98,8%
Dose:	25 µl solid test material
Treatment period:	3 minutes at room temperature or 1 hour in the incubator
Positive control:	8N potassium hydroxide
Negative control:	Deionized water
Direct interaction with MTT:	Negative
GLP:	In compliance
Study period:	February 2017 – January 2018

From the day of arrival in the laboratory, tissues were kept in the refrigerator. At least 1 hour, but not more than 1.5 hours before test substance application, tissues were transferred to 6-well plates with 0.9 ml assay medium and preconditioned in the incubator at 37°C. The preincubation medium was replaced by fresh medium immediately before application. 25 µl deionized water was applied first. Thereafter, a bulk volume of ca. 25 µl solid test material (about 26 mg) was applied with a sharp spoon and homogeneously distributed with the water. Control tissues were concurrently treated with 50 µl of deionized water (NC) or with 50 µl of 8N potassium hydroxide (PC). The tissues were washed with PBS to remove residual test material 3 minutes or 1 hour after start of the application treatment. Rinsed tissues were kept in 24-well plates (holding plates) at room temperature on assay medium until all tissues per application time were dosed and rinsed. The assay medium was then replaced by MTT solution and tissues were incubated for 3 hours. After incubation, the tissues were washed with PBS to stop the MTT incubation. The formazan that was metabolically produced by the tissues was extracted by incubation of the tissues in isopropanol. The optical density at a wavelength of 570 nm (OD570) of the extracts was spectrophotometrically determined. Blank values were established of 4 µl wells filled with isopropanol for each microtiter plate. The mean viability of the tissues treated with benzophenone-4 determined after an exposure period of 3 minutes was 104.0% (individual viability values: 106.8% and 101.1%) and 42.6% (individual viability values: 52.4% and 32.9%) after an exposure period of 1 hour. The variability between the tissues (CV of % viability) of the test substance at the 1-hour exposure is 32.4% and therefore out of the acceptance range. However, as all other quality criteria of the test were met and both viability values are well above the cutoff for skin corrosion, this deviation is not considered to adversely affect the result of this study. Based on the results observed and by applying the evaluation criteria, it was concluded that benzophenone-4 is not corrosive under the test conditions chosen.

(Remmele 2018a)

In another study, following the OECD TG 431 guideline, 25 mg of neat benzophenone-4 was applied evenly to the surface of a 3D human EpiDerm™ model (surface of 0.5 cm^2), for either 3 or 60 minutes. Prior to use, the tissues were incubated (37 ± 1°C, 5 ± 1% CO_2) in 0.9 ml of fresh maintenance medium in 6-well plates for ~1 hour. Before dosing, the tissues were moistened with 25 µl of sterile water to improve the contact of the tissue surface to the test article. Approximately 25 mg of solid test article was evenly applied to the apical surface of each tissue. Each treatment with test article or control was conducted in duplicate. The exposure period for the test articles and controls was 3 and 60 minutes. For the 60-minute exposure, the dosed tissues were placed in an incubator at 37 ± 1°C, 5 ± 1% CO_2 for the remainder of the 60-minute exposure period. Following the rinsing period, the MTT assay was performed by transferring the tissues to 24-well plates containing 300 µl MTT medium (1.0 mg/ml). After 3 hours of incubation at 37 ± 1°C, 5 ± 1% CO_2 in a humified incubator, the blue formazan salt was extracted by submerging the tissues in 2 ml isopropanol in a 24-well plate. The extraction time was approximately 2 hours and 5 minutes with gentle shaking. The optical density of the extracted formazan (200 ml/well if a 96-well plate) was determined at 570 nm. Relative cell viability was calculated for each tissue as % of the mean negative control tissue.
The mean of OD for the test chemical was determined to be 2.098 and 0.315 for the 3 minutes endpoint and 1 hour endpoint, respectively. The mean % tissue viability compared to negative control (n=3) of the test chemical was determined to be 85.7 % and 13.4 % for the 3 minutes endpoint and 1 hour endpoint, respectively. Based on these values, benzophenone-4 can be

considered to be corrosive to skin. It can be further classified under the category "Category 1" as per CLP Regulation.

(ECHA dossier cited in CIR 2021)

SCCS comment

Based on available *in vitro* data, it cannot be excluded that benzophenone-4 may have a corrosive effect after prolonged exposure.

***In Vitro* Skin Irritation: Reconstructed Human Epidermis (RhE)Test Method**

Guideline:	OECD Test Guideline 439 (July 2015)
Test system:	EpiDerm™ 200 model (0.6 cm²)
Number of replicates:	Three tissues per condition
Test substance:	Benzophenone-4
Test substance No.:	02/0296-4
Test item:	Neat
Batch:	Y7115C182
Purity:	98,8%
Dose:	25 µl solid test material
Treatment period:	1 hour
Post-treatment incubation time:	42 hours
Positive control:	5% (w/v) sodium dodecyl sulphate (SDS) in water
Negative control:	Sterile PBS
Direct interaction with MTT:	Negative
GLP:	In compliance
Study period:	February 2017 – January 2018

On the day of arrival in the laboratory, the tissues were transferred to sterile 6-well plates with 0.9 mL assay medium and preconditioned in the incubator at 37°C. After 1 hour, the pre-incubation medium was replaced by fresh medium and preconditioning continued for 18 ± 3 hours. 25 µl sterile PBS was applied first. Thereafter, a bulk volume of ca. 25 µl solid test material was applied with a sharp spoon and homogeneously distributed together with the fluid. Control tissues were concurrently treated with 30 µl sterile PBS (NC) or with 30 µl 5% SDS (PC). A nylon mesh was carefully placed onto the tissue surface of the NC and PC afterwards. The tissues were kept under the laminar flow hood at room temperature for 25 minutes overall and for 35 minutes in the incubator. The tissues were washed with sterile PBS to remove residual test material 1 hour after start of application. Rinsed tissues were blotted on sterile absorbent paper and transferred into new 6- well plates pre-filled with 0.9 ml fresh medium. When all tissues were rinsed the surface of each tissue was carefully dried with a sterile cotton swab. Subsequently, the tissues were placed into the incubator at 37°C for 24 ± 2 hours. After 24 ± 2 hours, the tissues were transferred into new 6-well plates pre-filled with 0.9 ml fresh medium and placed into the incubator for an additional 18 ± 2-hour post-incubation period. After the post-incubation period, the assay medium was replaced by 0.3 ml MTT solution and the tissues were incubated in the incubator for 3 hours. After incubation, the tissues were washed with PBS to stop the MTT incubation. The formazan that was metabolically produced by the tissues was extracted by incubation of the tissues in isopropanol. The optical density at a wavelength of 570 nm (OD570) of the extracts was spectrophotometrically determined. Blank values were established of 4 microtiter wells filled with isopropanol for each microtiter plate. The mean viability of the tissues treated with the test substance determined after an exposure period of 1 hour with 42-hour post-incubation, was 15.1% (individual viability values: 14.4%, 16.6% and 14.4%). Based on the results observed and by applying the evaluation criteria, it was concluded that benzophenone-4 shows a skin irritation potential under the test conditions chosen.

(Remmele M. 2018a)

SCCS comment
The report does not provide clear data regarding direct reduction of MTT. According to the guideline, if there is evidence of direct MTT reduction, additional measures should be implemented to detect and account for potential interference.

Animal *in vivo* data:

-Single application:

At concentrations up to 16%, benzophenone-4 was non-irritating when applied to rabbit skin in petrolatum. It was non-irritating at 8% in dimethylphthalate (DMP) and minimally irritating in this vehicle at 16%. When a 5% aqueous solution was used, no irritation was observed in rabbits.

(SCCNFP/0085/98; CIR 1983)

-Repeated application:

Marzulli and Maibach conducted a 16-day cumulative irritation test on six New Zealand albino rabbits to evaluate the skin irritation potential of a benzophenone-4 solution. The rabbits' depilated backs were exposed to alcohol-based solutions containing either 10% or 1% of benzophenone-4. The exposed sites were scored for irritation 24 hours after each application, and the same procedure was repeated every other day for five weeks, resulting in a total of 16 applications. The researchers calculated the average cumulative irritation score, with a maximum possible score of 64, and found that the application of 10% and 1% benzophenone-4 solutions resulted in irritation scores of 3.6% and 0.3%, respectively. The study showed that a concentration of 10% benzophenone-4 caused minimal irritation in rabbits.

(Marzulli and Maibach 1975)

Human *in vivo* data:

- Single application:

An occluded patch test with benzophenone-4 applied in concentrations of 4%, 8% and 16% in dimethylphtalate and petrolatum was carried out in 14 human subjects. The duration of exposure for this test is not stated. There was no reaction in any of the subjects at the 4% concentration. At the 8% concentration, 1/14 subjects showed signs of irritation, and at 16% 4/14 subjects showed irritation. Benzophenone at 4% and 8% were not irritating in neither vehicle.

(SCCNFP/0085/98)

The incidence of irritant reactions caused by benzophenone-4 was investigated using a group of 80 participants. Different concentrations of benzophenone-4 (2%, 5% and 10%) in petrolatum were applied to the skin using Finn chambers of 8 mm in diameter. The patches were secured with adhesive tape and left on the upper back for a duration of 2 days. The reactions observed were evaluated based on the criteria established by the International Contact Dermatitis Research Group (ICDRG). Benzophenone-4 at 2%, 5% and 10% in petrolatum led to irritant reactions in 1, 4 and 6 subjects, respectively, indicating that the test substance is moderately irritating to the skin under the chosen testing conditions.

(Kerr *et al*. 2009)

- Repeated application:

Marzulli and Maibach conducted a 21-day test on six adult white humans to determine the skin irritation potential of a benzophenone-4 solution. The subjects were exposed to an

alcohol-based solution containing either 1% or 10% of benzophenone-4, and the exposed sites were scored for irritation after 24 hours. The same procedure was repeated every other day, three days per week for seven weeks, resulting in a total of 21 applications. The researchers calculated the average cumulative irritation score, with a maximum possible score of 84, and found that the application of 10% and 1% benzophenone-4 solutions resulted in irritation scores of 8.6% and 53.1%, respectively. This suggests that benzophenone-4 exhibited irritant characteristics at the 10% dose.

(Marzulli and Maibach 1975)

A Shelanski repeated insult patch test was carried out in 50 subjects, using a 5% aqueous solution at pH 7. A volume of 0.5 ml of the solution was applied to the skin for 24 hours, and then evaluated. This procedure was repeated every 48 hours until 15 applications had been made. The findings of this study revealed no evidence of irritation among the subjects throughout the entire testing period.

(Anon 1978 from SCCNFP/0085/98)

A further repeat insult patch test (modified Draize Shelanski method) was conducted in 100 healthy subjects in order to evaluate the irritancy and sensitising property. A test formulation containing benzophenone-4 at a 25% concentration in petrolatum was applied for a period of 24-hours, followed by a 7-day rest period. Subsequently, challenge patches with a 10% concentration in petrolatum were applied for 24 hours. None of the 100 subjects displayed any reactions to either the repeated applications or the challenge application.

(Kligman 1976)

SCCS comment
Skin irritation was measured in the same test as that used for skin sensitisation (repeat insult patch test according to Draize Shelanski). SCCS has expressed its ethical concerns about conducting human skin sensitisation tests, such as the HRIPT (SCCNFP/0120/99).

Conclusion from the applicant
Neat benzophenone-4 has been classified as Category 1 corrosive based on *in vitro* assay data. In rabbits, it is minimally irritating at a concentration of 16%, and not irritating at a concentration of 8%. Concentrations above 5% led to mild observations of skin irritation in human subjects.

SCCS overall conclusion on skin irritation
- *In vitro* data using human reconstructed skin models indicate a corrosive effect of neat benzophenone-4 after 1 hour exposure, as expected from the acidic pH of the sulfonic acid-containing substance.
- Historical *in vivo* animal data indicate minimal irritation of benzophenone-4 in dimethylphthalate at 16% upon single application, but no irritation was observed in rabbits when a 5% aqueous solution was used. Repeated application of an alcohol-based solution containing 10% benzophenone-4 to rabbit skin caused minimal irritation.
- Historical *in vivo* human data indicate that single application of benzophenone-4 at 16% in dimethylphtalate and petrolatum results in mild irritation, and is moderately irritating above 5% in petrolatum. Repeated application of benzophenone-4 in an alcohol-based solution exhibited irritant characteristics at the 10% dose.
- Skin irritation was measured in the same test used for human skin sensitisation using a repeat insult patch test according to Draize Shelanski, which is considered as non-ethical (SCCNFP/0120/99).

3.4.1.2 Mucous membrane irritation / eye irritation

In vitro data:

Bovine Corneal Opacity and Permeability Test Method

Guideline:	OECD Test Guideline 437 (July 2013)
Test material:	Bovine cornea
Replicates:	3 corneas per condition
Test substance:	Benzophenone-4
Test substance No.:	02/0296-4
Test item:	20% (w/v) solution in deionized water
Batch:	Y7115C182
Purity:	98,8%
Test volume:	750µl
Treatment period:	4 hours
Post-treatment incubation time:	/
Positive control:	20% (w/v) imidazole solution in deionized water
Negative control:	Deionized water
GLP:	In compliance
Study period:	February 2017 – December 2017

Corneas free of defects (opacity, scratches, pigmentation etc.) were dissected with a 2 to 3 mm rim of sclera. Isolated corneas were mounted in cornea holders that consists of anterior and posterior chambers. Both chambers were filled to excess with pre-warmed Eagle's MEM (without phenol red) and then equilibrated in a vertical position at about 32°C for at least 1 hour. After the equilibration period, the medium in both chambers was replaced by fresh pre-warmed medium and initial corneal opacity readings were taken for each cornea with an opacitometer. Any corneas that showed macroscopic tissue damage or an opacity value < 550 opacity units were discarded. The remaining corneas were then distributed into negative control, positive control and treatment groups. Each corneal holder was uniquely identified with a number on the chambers. Before application, the medium in the anterior chamber was removed by using a syringe. 750 µl of the 20% (w/v) test-substance preparation (non-surfactant) were applied into the anterior chamber by using a pipette. For the control tissues, 750 µl deionized water (negative control, NC) or 750 µl 20% (w/v) solution of imidazole in deionized water (positive control, PC) were applied into the anterior chamber by using a pipette. The corneas were incubated in a horizontal position at about 32°C for approximately 4 hours (non-surfactant solids). The test substance, the NC and the PC were then removed from the anterior chamber by using a syringe and the epithelium was washed at least 3 times with Eagle's MEM (containing phenol red) and once with Eagle's MEM (without phenol red). Both chambers were then refilled with fresh Eagle's MEM (without phenol red). Before measurement, each cornea was visually observed and observations were recorded. Final corneal opacity readings were taken for each cornea with an opacitometer. For determination of permeability, the medium in the anterior chamber was replaced by 1 ml sodium fluorescein solution (5 mg/ml for solid test substances) and incubated for 90 ± 5 minutes in a horizontal position at about 32°C. The amount of sodium fluorescein that permeated through the corneas into the posterior chamber was spectrophotometrically measured. Three aliquots per cornea were transferred to a 96-well microtiter plate and the optical density (OD490) was determined. An aliquot was diluted 1:5 in Eagle's MEM (without phenol red) and analogously measured (PC, only). Both opacity and permeability measurements were used to calculate an *In Vitro* Irritancy Score (IVIS). Two test runs of the BCOP test were performed. The 1st test run resulted in a borderline finding of benzophenone-4 treated corneas with a mean IVIS score of 61.0 (mean opacity value = 61.0; mean permeability value = 0.000). The mean IVIS value of 75.9 (mean opacity value = 75.9; mean permeability value = 0.000) obtained in the 2nd test run showed a clearly positive result for benzophenone-4. Based on these results it was concluded that benzophenone-4 causes ocular corrosion or severe irritation.

(Remmele 2018b)

Following the OECD TG 492 guideline, 50 mg of neat benzophenone-4 was applied to the surface of a 3D human MatTek EpiOcular™ model (surface of 0.5 cm^2). The tissues were exposed to the test article for approximately 6 hours ± 15 minutes for solid test articles and controls at approximately 37°C, 5% CO_2 in a humidified incubator. After the exposure, the test article was rinsed off the tissues and the tissues were soaked in media for ~25 minutes for solid test articles and controls. Following the washing and post soak, the tissues were rinsed and incubated at approximately 37°C, 5% CO_2 in a humidified incubator for a post-exposure recovery time of 18 hours for solid test chemicals and controls. Tissue viability was assessed by 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The mean % tissue viability of the test substance was determined to be 3.6%. Based on these values, it was concluded that benzophenone-4 has the potential to induce eye irritation.
It can be further classified as ''Irritating to eyes in Category 2" as per CLP Regulation.

(ECHA dossier cited in CIR 2021)

In vivo animal data:

In an *in vivo* eye irritation study in albino rabbits with limited documentation and not according to current standard protocols, benzophenone-4 has been instilled into the conjunctival sac of one eye at a concentration of 5% in water. Six rabbits were treated without washing out the eye and the treated eye of 3 additional rabbits was washed out 4 seconds after test material instillation. None of the nine rabbits dosed including six with unwashed eyes and three with washed eyes showed any involvement of the cornea, or the iris, or the conjunctiva at any time during the seven days after being dosed.

(IBL 1962 cited in CIR 1983)

In an additional *in vivo* eye irritation study in rabbits, reported in a secondary source with limited documentation and not according to current standard protocols, benzophenone-4 has been instilled into the conjunctival sac of one eye at concentrations of 4%, 8% and 16% in dimethylphtalate and petrolatum. Six rabbits were treated without washing out the eye. Corneal effects were observed after instillation of 16% and mild conjunctivitis was observed after instillation of 8% benzophenone-4 in both vehicles. These findings indicate an eye irritation potential of benzophenone-4 at concentrations > 8%.

(IBL 1967 cited in CIR 1983)

In an overall safety assessment of benzophenone-4, an *in vivo* study with 6 rabbits according to Draize not according to current guidelines has been reported from secondary source with limited documentation. Undiluted (100%) benzophenone-4 was instilled and average score for day 1, 2 and 3 was 2.58, 2.38 and 2.05, respectively. No further data on later observation timepoints has been given. Benzophenone-4 was determined by the authors to be irritating to iris and conjunctiva. No further data were provided in terms of specific corneal, iris and conjunctival scores of single animals to compare these findings with the classification criteria according to CLP.

(ACC 1959 cited in CIR 1983)

The details of the 4 rabbit eye irritation tests for benzophenone-4 are summarized in Table 3.

Table 3: Rabbit eye tests for benzophenone-4.

Test material and method	Average Score	Results	Reference
0.1ml of 4,8,or 16% BP-4 in DMP (FHSLA method)	NR	Irritating (cornea, conjunctiva at 16%; conjunctiva at 8%)	IBL study (April 1967) as cited in CIR 1983
0.1ml of 4,8,or 16% BP-4 in petrolatum (FHSLA method)	NR	Irritating (cornea, conjunctiva at 16%; conjunctiva at 8%)	IBL study (April 1967) as cited in CIR 1983
0.1 ml of 5% BP-4 in water (Draize test)	0	Non-irritating	IBL study (April 1962) as cited in CIR 1983
100mg of 100% BP-4	2.36	Irritating to iris and conjunctiva	ACC study (April 1959) as cited in CIR 1983

SCCS overall conclusion on eye irritation
According to *in vitro* (MatTek EpiOcular™ model) and historical *in vivo* animal data, undiluted benzophenone-4 is irritating to the eyes. Benzophenone-4 was irritating to the rabbit eye at concentrations of 8 and 16% in dimethylphtalate and petrolatum. Benzophenone-4 is not irritating to the rabbit eye at 5% in water. However, *in vitro* BCOP data indicate that 20% (w/v) in deionized water is corrosive, due to the acidic pH of the sulfonic acid-containing substance. Therefore, without further test results, it cannot be excluded that benzophenone-4 in the currently allowed concentration is not irritating to the eyes.

3.4.2 Skin sensitisation

In vivo animal data:

Guideline:	OECD TG 406
Method:	Buehler test
Species:	Guinea pig (Pirbright White Dunkin Hartley)
Induction:	3x50% in aqua bidest (once per week)
Challenge:	5% (anterior flank) & 50% (posterior flank) in aqua bidest
Vehicle:	Aqua bidest
No. of animals/group:	20
Test substance:	benzophenone-4
Batch No.:	2404202
Purity:	97%
Dose:	0.5 ml
Positive control:	Alpha-Hexylcinnamaldehyde techn.
Study period:	June 1996 – July 1996

The Buehler test was conducted to evaluate the potential sensitizing effect of benzophenone-4, on guinea pig skin. The study consisted of a pretest and a main test. During the pretest, different concentrations of benzophenone-4 were applied to the guinea pigs' skin, up to a maximum of 50%, and no signs of skin irritation were observed. Based on these results, appropriate concentrations for the main test were determined. The main test involved a test group of 20 guinea pigs and two control groups, each consisting of 10 animals. The control groups were included to rule out the possibility of any primary skin irritation caused by the substance. The test group animals underwent three inductions with benzophenone-4, with a weekly application to the same area of the skin. The first two inductions using 50% preparations of the test substance did not produce any signs of skin irritation. However, after

the third induction, one out of the 20 animals exhibited well defined erythema on the skin. This particular animal also showed varying degrees of erythema, ranging from well-defined to very slight, at 24 and 48 hours, respectively, following the application. Additionally, a very slight swelling (edema) was observed only 24 hours after removing the patch during the challenge application. The control animals did not receive any benzophenone-4 treatment. The incidence of skin findings in the animals did not surpass 15 percent, which was the predetermined threshold for determining sensitization according to the evaluation criteria of the study. Therefore, based on the evaluation of the results and adherence to the study's criteria, it was concluded that under the selected test conditions, benzophenone-4 does not possess a sensitizing effect on the skin of guinea pigs.

(Polloth and Hellwig 1997)

Guideline (s)/Study:	OECD TG 406/Commission Directive 92/69/EC
Method:	Guinea Pig Maximation Test (GPMT)
Species:	Guinea pig (Pir bright White Dunkin Hartley)
Induction:	Intradermal: injections of 5% in oil/water emulsion/ Freund's adjuvant or 0.9% aqueous NaCl solution versus controls (1/1 ratio) Percutaneous: 10% in oil/water emulsion, versus controls
Challenge:	5% in oil/water emulsion
Vehicle:	Oil/water emulsion
No. of animals/group:	10 (test group) + 5 (control group)
Test substance:	Benzophenone-4
Batch No.:	Abl.-No. 27-03402
Purity:	98%
Dose:	0.5 ml
Duration:	24 hours
Positive control:	Alpha-Hexylcinnamaldehyde techn.
Year:	1998

The study consisted of a pretest to determine suitable concentrations for the main test, followed by the induction and challenge phases. In the pretest, the minimum irritant concentration was found to be a 10% formulation of the test substance in an oil/water emulsion, while the maximum non-irritant concentration was determined to be a 5% formulation in the same emulsion. The main test involved both intradermal and percutaneous induction methods. The animals received 5% intradermal injections on their shoulders using either an oil/water emulsion or a mixture of Freund's adjuvant or 0.9% aqueous NaCl solution in a 1:1 ratio. Control groups were included, receiving either an empty formulation or Freund's adjuvant. Skin reactions were evaluated 24 hours after application. Percutaneous induction was conducted by applying gauze patches containing the test substance formulation at a concentration of 10% to the flank skin under an occlusive dressing. Control groups were received patches with the empty formulation. Readings were taken 48 hours after application. Following the induction phase, two challenges were performed. The flank skin of the guinea pigs was exposed to gauze patches containing the test substance formulation at a concentration of 5% for 24 hours during each challenge. Readings were taken 24 and 48 hours after removing the patches. Based on the study findings and the evaluation criteria, it was concluded that benzophenone-4 exhibits a sensitizing effect on the skin of guinea pigs in the Maximization Test under the chosen test conditions.

(Kühlem and Hellwig 1998)

Human data:

In a human repeated insult patch test, 5% benzophenone-4 in water has been applied topically for 24 hours to 50 volunteer subjects. After 15 applications and a 2-week rest period, a final 5% benzophenone-4 challenge application was applied again for a 24-hour period.

None of the fifty subjects reacted to any of the 15 applications or to the challenge application. From the results obtained in this study, a 5% benzophenone-4 solution in water was found to be neither a primary irritant, a fatiguing agent nor did it produce any skin sensitization under the chosen testing conditions.

(GAF Corp 1962)

In another human repeated insult patch test, benzophenone-4 has been topically applied to 100 healthy male and female subjects at a concentration of 25% in petrolatum for 10 alternate-day 24 hour periods under occlusion. Following a 7-day rest period, challenge patches with 10% benzophenone-4 in petrolatum were applied in the same manner to fresh sites on the backs or volar forearms of all 100 subjects for 24 hours. Challenge sites were read on removal of the patch and 24 hours thereafter. None of the 100 subjects reacted to any of the 10 applications or to the challenge application. Therefore, no instances of skin irritation or skin sensitization were observed after application of benzophenone-4 under the chosen testing conditions.

(Kligman 1976)

SCCS comment
The forementioned studies are not comprehensive investigations but rather concise summaries outlined in a brief paragraph within the applicant dossier, and the original study reports were not provided for SCCS evaluation.
The repeat insult patch test is considered as non-ethical (SCCNFP/0120/99).

Other observations from the published literature related to human skin sensitisation of benzophenone-4 provided by the applicant are shown in Table 4.

Table 4: Human observations on allergenicity for benzophenone-4 from published literature.

Method details	Observations	Reference
Not stated. 15 human subjects (4 males, 11 females) had reacted to sunscreens. Of these, 8 had used sunscreens before occasional sun exposure, and 6 had used them regularly for chronic lupus erythematosus, melasma, vitiligo, rosacea, drug photosensitivity, and atopic dermatitis. Patch testing (procedure not stated) performed.	4 allergic contact dermatitis reactions to Benzophenone-4	Goncalo *et al.* 1995
10% in petrolatum. 402 human subjects with suspected clinical photosensitivity. Patch and photopatch tests performed according to ICDRG guidelines. UVA dose of 5 or 10 J/cm^2 used for photopatch testing.	No allergic reactions	Schauder & Ippen 1997

5% and 10% in white paraffin. 1155 human subjects (from 17 centres across the UK, Ireland and the Netherlands). Photopatch testing (over 2-year period) involved application of test substance (on aluminium Finn chamber) to skin of mid-upper back (paravertebral area avoided) for 24 hours or 48 hours (depending on the centre). Contact dermatitis units traditionally applied allergens for 48 hours, and photobiology units traditionally applied allergens for 24 hours. Following patch removal, one set (dark control) covered with light-impermeable occlusive dressing, and the other set irradiated with fluorescent UVA (5 J/cm^2). Reactions scored at 48 hours post-irradiation, and, if possible, at 24 hours and 72 hours. ICDRG visual scoring system was used.	Photoallergy in 2 and 5 patients, in 5 and 10% dose groups, respectively. Contact allergy in 2 and 9 patients, in 5 and 10% dose groups, respectively. Irritation reactions observed in 2 and 4 patients, in 5 and 10% dose groups, respectively.	Schauder & Ippen 1997
Not stated. 160 human subjects (37 male, 123 female). Retrospective chart review on patients who underwent photopatch testing in Canada between January of 2001 and December of 2010. Duplicate sets of allergens applied to back. At 24 hours, 1 set of allergens uncovered and exposed to UVA at a dose of 5 J/cm^2. Other set of allerge shielded from UVA exposure. 24-hour reactions to non-irradiated compounds assessed at 15 to 20 minutes later. On following day, irradiated patches read at 24 hours post-irradiation. Reactions at non-irradiated patch test sites read 48 hours after application.	Benzophenone-4 caused allergic contact dermatitis in 3 patients, but did not cause photoallergic reactions.	Rodriguez *et al.* 2006
10%. 553 human subjects. Over a period of 3 years, patients were patch tested (Finn chambers) with each test substance. Results recorded at 48 hours (day 2) and 96 hours (day 4). Positive reactions (+ to +++) graded according to international recommendations (not specified).	13 patients with positive reactions to Benzophenone-4.	Hughes & Stone 2007
10% in petrolatum. 1527 human subjects. A retrospective analysis involved the reviewing of 1527 charts in the University of British Columbia Contact Dermatitis Clinic patch test database from January of 2009 to July of 2012. 23 of the patients tested with the sunscreen series at the clinic. All 1527 patients patch tested with 70 allergens on NACDG screening series. Patch test chambers containing test substance applied to upper back and secured with tape for 48 hours. Reactions scored (using the ICDRG grading scale) at time of patch removal and at 96 hours to 120 hours.	Of the 23 patients tested, 1 had a positive contact allergy reaction.	Beleznay *et al.* 2014
10% (in petrolatum). 4857 human subjects. NACDG study. Patch testing performed using Finn chambers.	Positive reaction rate of 2.1% (100 allergic reactions). Values for clinical relevance of allergic reactions: definite relevance (0), probable relevance (20 of 100 patients (20%)), possible relevance (53 of 100 patients (53%)), and past relevance (9 of 100 patients (9%)).	DeKoven *et al.* 2017

10% in petrolatum. 5592 human subjects tested. NADCG study. Patch testing with Finn chambers.	Of the 5592 patients, 93 had a positive (allergic) reaction. Values for clinical relevance of allergic reactions were: definite relevance (3 of 93 patients (3.2%)), probable relevance (12 of 93 patients (12.9%)), possible relevance (45 of 93 patients (48.4%)), and past relevance (8 of 93 patients (8.6%)).	DeKoven *et al.* 2018
2% in petrolatum. 1390 human subjects tested with Benzophenone-4. British Society for Cutaneous Allergy (BSCA) retrospectively reviewed results from their facial patch test series. Review involved 12 centers in United Kingdom and Ireland for 2-yr period (January of 2016 to December of 2017).	Of the 1390 patients patch tested with Benzophenone-4 (2% in petrolatum), 0.79% (confidence interval (CI): 0.44% to 1.41%) had allergic reactions.	Rolls *et al* 2021

Information provided to ECHA under CLP notifications has indicated that benzophenone-4 causes skin sensitization (H317, skin sens.1).

Conclusion from the applicant

The results from the Guinea-pig data (GPMT positive vs Buehler negative results) suggest that when the skin barrier is breached e.g., when using intradermal injection in a GPMT, sensitisation is possible, but generally the skin barrier is good at preventing benzophenone-4 absorption across the skin. In humans, there are incidences of benzophenone-4 contact allergy, but benzophenone-4 is not a common allergen of concern.

SCCS overall conclusion on skin sensitisation

- For the Buhler test, which usually has a low sensitivity, a high induction was used but since the dermal absorption was low, the exposure was still limited and the Buhler test was negative.
- For the Maximisation test, the exposure to the compound is higher than in the Buhler test because of exposure after injection and a positive result was shown.
- According to ECHA, benzophenone-4 is classified as skin sens.1.
- Human data from published literature studies show that benzophenone-4 has skin sensitising properties. The old study summaries provided are incomplete and non-conclusive.
- The allergy patch testing studies on human subjects are considered as non-ethical (SCCNFP/0120/99) and were of limited value for the current assessment due to:
  - lack of clarity regarding the test substance, making it difficult to determine whether the test substance was based on the product or the individual ingredient
  - undefined concentration of the ingredient
  - absence of method description
  - no full study reports.

In view of the limited number of reported cases in the literature compared to the potential exposure of many individuals, the risk of sensitisation to benzophenone-4 can be regarded as very low.

3.4.3 Acute toxicity

3.4.3.1 Acute oral toxicity

Benzophenone-4 was evaluated for acute oral toxicity using 20 rats (strain not stated). The test substance was administered orally by gavage in doses ranging from 1250 to 10000 mg/kg/day, suspended in agar/tween. After dosing, the rats were observed for 7 to 14 days, during which ataxia (lack of coordination) was observed as a clinical sign. The study reported an oral LD50 of 3530 mg/kg/day.

(CIR 1983; CIR 2021)

Benzophenone-4 was tested for acute oral toxicity in Sherman Wistar rats using a 5% solution in water administered by gavage at doses ranging from 200-6400 mg/kg/day (it was not specified if the doses refer to the active ingredient). The study, conducted in 1962, had limited documentation, but no deaths were observed at the highest dose, and an LD50 value greater than 6400 mg/kg/day was established. However, since it was not specified whether the doses refer to the active ingredient benzophenone-4 or the solution, it cannot be ruled out that an LD50 value greater than 320 mg benzophenone-4/kg bw was obtained.

The applicant mentions also two acute oral toxicity studies conducted in rats to evaluate the toxicity of benzophenone-4. In the first study, the substance was administered via gavage to 20 rats at doses ranging from 1250-10000 mg/kg/day in Agar/Tween. The LD50 was determined to be 3520 mg/kg/day, and the substance was classified as slightly toxic. In the second study, 15 rats were administered benzophenone-4 via gavage at doses ranging from 2500-10000 mg/kg/day in Water/Agar/Tween. The LD50 was determined to be 6150 mg/kg/day, and the substance was classified as practically non-toxic.

Based on the available data, it can be concluded that benzophenone-4 is practically non-toxic when administered orally as a single dose.

(GAF Corp 1962)

SCCS comment
Original study reports were not available, but the studies cited point to low or no acute oral toxicity of benzophenone-4 in rats.

3.4.3.2 Acute dermal toxicity

A study conducted in 1983 evaluated the acute dermal toxicity of benzophenone-4 on 10 albino rabbits, using a dose of 5000 mg/kg/day. The test substance was applied to the epilated skin of the back or flanks and left in contact for 18-24 hours. No mortality was observed even at the highest dose of 5000 mg/kg/day. The study found no evidence of systemic toxicity or irritation and reported a dermal LD50 value greater than 5000 mg/kg/day for benzophenone-4.

(CIR 1983)

SCCS comment
The original study report was not provided for SCCS evaluation, but the results cited point to no acute dermal toxicity.

3.4.3.3 Acute inhalation toxicity

/

SCCS comment
Original reports for acute oral, dermal and inhalation acute toxicity on benzophenone-4 were not made available to the SCCS. However, in the SCCS Notes of Guidance (SCCS1647/22), the following is indicated:
'In light of the animal testing ban for cosmetic ingredients, data on acute toxicity is not mandatory for assessing the safety of cosmetic ingredients for consumer use. A WoE approach may be sufficient'.

3.4.4 Repeated dose toxicity

3.4.4.1 Repeated dose (28 days) oral / dermal / inhalation toxicity

/

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

Guideline:	OECD TG 408
Species/strain:	Rat/Wistar
Group size:	10 male and 10 female rats
Test substance:	Benzophenone-4
Batch:	2404202
Purity:	98.3%
Vehicle:	None, mixed in food
Dose levels:	750, 3000 and 15000 ppm (58, 230 and 1239 mg/kg/day)
Route:	Oral
Administration:	Diet
GLP:	Yes
Study period:	April 1995 – April 1996

In a 90-day repeated dose study conducted on Wistar rats, the toxicity of benzophenone-4 was investigated. Rats were fed 750, 3000 and 15000 ppm (equivalent to 58, 230 and 1239 mg/kg/day) doses of benzophenone-4 for 90 days. The rats were closely monitored for signs of toxicity. The study found that benzophenone-4 did not have any adverse effects on body weight, food consumption, or urinalysis. Although there were some changes in urea levels in the blood of male and female rats, these changes were not considered to be adverse. Male rats exposed to the highest dose showed a decrease in body and liver weight, while female rats exposed to the middle dose showed an increase in adrenal gland weight. However, these changes were not associated with any adverse gross or histopathological findings. Overall, the study concluded that benzophenone-4 did not have significant toxic effects on the rats, as there were no adverse findings in the clinical examination or histopathological analysis. Therefore, the highest dose of 1239 mg/kg/day was taken as the NOAEL.

(BASF AG 1996)

SCCS comment
Original study reports were not provided with the first submission, but the original study report of the 90-day repeat dose toxicity study was requested and received. A NOAEL of 1239 mg/kg/day was derived. The study report was not signed.

3.4.4.3 Chronic (> 12 months) toxicity

/

3.4.5 Reproductive toxicity

3.4.5.1 Fertility and reproduction toxicity

/

3.4.5.2 Developmental Toxicity

Guideline:	OECD TG 422
GLP:	/
Species/strain:	Rat/Wistar
Group size:	26/group (13M + 13F)
Test substance:	Benzophenone-4
Batch:	2404202
Purity:	98.3%
Vehicle:	Corn oil
Dose level:	750, 1000, and 1250 mg/kg/day
Dose volume:	2ml/100g body weight
Administration:	Gavage
Study period:	2018 (cited in the EU REACH record)

The test substance was administered orally (in corn oil, by gavage) at doses of 750, 1000, and 1250 mg/kg/day. Male Wistar rats (n=13) were treated 2 weeks before mating and thereafter for a total of 48 dosing days. Female Wistar rats (n=13) were treated 2 weeks before mating, and during mating, gestation, and lactation, for a total of approximately 63 days of dosing. Recovery groups of males and females (n=5 per dose) were observed for 2 weeks after the final dose.

No morbidity was observed during the treatment periods. Some incidental effects on body weight were observed that were not considered treatment related. Hormonal data showed no significant effects on T4, TSH, testosterone(males) and estradiol (females). No notable effects on organs or blood chemistry parameters were observed. Pregnancy rates, gestation length and litter size were not affected by treatment. No significant effects were seen on the number of live births, pup weight, pup survival or sex ratio. No test substance abnormalities were seen in pups. Special investigations on thyroid and parathyroid glands revealed no abnormalities.
Based on the results reported, a NOAEL of 1250 mg/kg/day for male and female rats was considered.

(Sustainability Support Services (Europe) AB 2018)

SCCS comment
The original study report was not provided with the initial submission, but was sent upon request. A number of deviations from OECD TG 422 were observed:
- the study employed a dose volume of 2 ml/100g body weight, while the recommended guideline suggested using only 1 mg/100g body weight (with 2 ml being acceptable for aqueous solutions). Here corn oil has been used.
- the report did not include information on the vaginal smear examination to be conducted on the day of necropsy, as recommended in the guideline.

Additionally, there is an inconsistency in the report where one male subject in the high-dose group was reported as deceased, but this information does not align with the subsequent data presented in the table 11, appendix 11, or the histopathology report (appendix 6).
It is possible that a typographical error exists in the report, which references "post dosing oestrous cycle examination", where the guideline recommends pre-treatment evaluation of oestrous cyclicity.

Other studies:

The results of other studies on the toxicity to reproduction are summarised in Table 5.

1 **Table 5: Studies on the toxicity to reproduction (other studies).**
2

Method details	Observations	Reference
Rat (Wistar [rat] - CrlGlxBrlHan:WI) male toxicity to reproduction: other studies oral: gavage () 1000mg/kg bw/day (nominal) 300mg/kg bw/day (nominal) Vehicle: corn oil Exposure: 10 days (daily) according to guideline OECD Protocol and Guidance for the Conduct of the Rodent Hershberger Assay; Phase 2 of the Validation of the Rodent Hershberger Assay	During clinical examinations no substance- related findings were observed. Concerning hormone investigations, co-administration of 1000 mg/kg bw/day of Benzophenone-4 and 0.4 mg/kg bw/day of Testosterone propionate did not affect the hormone levels of testosterone, dihydrotestosterone and luteinizing hormone in the serum of castrated male rats when compared with castrated animals given 0.4 mg/kg bw/day of testosterone propionate as reference androgen, only. Regarding pathology, the anti-androgen potential of a chemical is expressed in a deficient maturation of the accessory sex organs compared with the corresponding control. As expected, in castrated animals the absolute and relative weights of the accessory sex organs were significantly reduced when compared to castrated animals treated with Testosterone propionate, only. Histologically, prostate, seminal vesicle and bulbo-urethral gland were immature. After treatment with Benzophenone-4 in the presence of Testosterone propionate, the absolute and relative weights of the accessory sex organs were not significantly changed. Moreover, the histology of prostate, seminalvesicle and bulbo-urethral gland was comparable to the control group. No indications for an antiandrogen efficacy of Benzophenone-4 was determined.	BASF AG 2003
Rat (Wistar [rat] - Chbb : THOM (SPE)) female toxicity to reproduction: other studies oral: gavage () 500mg/kg bw/day (nominal) 1000mg/kg bw/day (nominal) Vehicle: water Exposure: 4 consecutive days (twice daily) equivalent or similar to guideline OECD 440: Uterotrophic Bioassay in Rodents: A short-term screening test for oestrogenic properties Study was conducted before OECD TG	A significant decrease in the body weight gain was observed in the dose groups 500 and 1000 mg/kg bw/d Benzophenone-4. However, in both test groups no clinical findings occurred in the course of the study, nor any substance related effects on the absolute or relative uterine weights of the animals were recorded. Benzophenone-4 did not promote uterine growth in immature Wistar female rats.	BASF AG 1998b

was available. The investigations described below were carried out in accordance with the Standard Operating Procedures (SOPs) of the laboratory units/departments involved in the study.		

SCCS comment

The *in vivo* Hershberger and uterotrophic studies on benzophenone-4 give negative results. These test results are described more extensively under 3.4.10.4. However, exposing the animals twice per day in the uterothropic assay is not in accordance with the OECD protocol.

3.4.6 Mutagenicity / genotoxicity

An *in vitro* battery of tests has been performed in order to assess the mutagenic/genotoxic potential of benzophenone-4. These studies are summarised and the results are described in the following sections.

3.4.6.1 Mutagenicity / genotoxicity *in vitro*

Three study reports on bacterial gene mutation, study report on mammalian gene mutation test, and on *in vitro* mammalian chromosomal aberration were provided by the applicant.

Bacterial gene mutation test 1

Guideline:	No OECD TG available
Test system:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, TA1538
Replicates:	Triplicate plates
Test substance:	Uvinul MS-40 (Lot #9082245); white powder
Batch:	/
Purity:	/
Solvent:	DMSO
Metabolic activ:	Aroclor induced rat liver S9 fraction
Positive controls:	N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 9-Aminoacridine (9-AA), 2-Nitrofluorene (2-NF) without S9-Mix; Aminoanthracene (2-AA) with S9 mix
Concentrations:	1000, 500, 100, 10, and 1 ug µg/plate with and without metabolic activation for all samples except for 400 which was tested at 500, 100, 10, 1, and 0.1 µg/plate. N-539 was tested at 1000, 500, 100, 10, and 1µg
Treatment:	Direct plate incorporation assay with incubation without and with S9-mix
GLP:	In compliance
Study period:	5 June – 20 June 1980

Benzophenone-4 solubilised in DMSO was tested for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 at multiple doses ranging from 1 to 1000 µg/plate, with and without Aroclor-induced rat liver microsomal S9 cell fraction. Dose levels were selected based on preliminary cytotoxicity studies.

A filter paper disc-agar overlay zone of inhibition method was used to give a preliminary indication of the toxicity of the test material for the test organism. All plates were incubated for 48 hours at 37°C after the agar overlay had hardened. The concentration of test material showing either no zone of inhibition or the smallest zone was then used as the basis for selection of doses to be used in the plate assay.

Results: The results of the negative as well as the positive controls performed in parallel fulfilled the acceptance criteria. In this study with and without S9 mix, the number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain. In addition, the positive control substances with and without S9 mix induced a significant increase in the number of revertant colonies. The results in the main study were evaluated against vehicle control data showing no increase in revertant colonies in any of tested concentrations.

Conclusion: Benzophenone-4 was non-mutagenic in all strains with and without metabolic activation up to 1000 µg/plate.

(Hill Top Research, Inc. 1980)

SCCS comment

The SCCS agrees with the conclusion of study report that benzophenone-4 was non-mutagenic in all strains with and without metabolic activation up to 1000 µg/plate. Historical negative control data were provided but not for positive controls. Purity of Uvinul samples is not provided.

Bacterial gene mutation test 2

Guideline:	OECD 471 (1983)
Test system:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537
Replicates:	Triplicate plates, two independent assays
Test substance:	UVINUL MS40: Yellowish powder
Batch:	Abl.-Nr. 58-0488
Purity:	> 98 .6%
Solvent:	Destil water
Metabolic activ:	Aroclor 1254 induced rat liver S9 fraction
Concentrations:	20, 100, 500, 1000, 2500 and 5000 µg/plate with and without metabolic activation
Treatment:	Direct plate incorporation assay with incubation without and with S9-mix
GLP:	In compliance
Study period:	19 July 1990 – 26 July 1990

Univul MS 40 was tested for induction of gene mutation in bacterial gene mutation test with Salmonella typhimurium strains TA 1535, TA 100, TA 1537, and TA 98.
The test was carried out in accordance with the OECD TG 471 and in compliance with GLP.

Uvinul MS 40 was tested using Standard Plate Test and Preincubation Test both with and without metabolic activation (rat liver S-9 mix) with doses 0, 20, 100, 500, 2500 and 5000 µg/plate. A precipitation was observed only after metabolic activation in the standard plate test at 5000 µg/plate and in the preincubation assay from about 2500 µg/plate onward. A bacteriotoxic effect was observed only in the preincubation test.

Parallel with each experiment with and without S-9 mix, a negative control (solvent control, sterility control) was carried out for each tester strain in order to determine the spontaneous mutation rate. As positive controls with S9-mix 2-aminoanthracene was used. Positive control without S9-mix N-methyl-N*-nitro-N-nitrosoguanidine (MNNG) for the strains TA 100 and TA 1535 and 4-nitro-o-phenylendiamine for the strain TA 98 and 9-aminoacridine chloride monohydrate for the strain TA 1537 were used.

Results: An increase in the number of his4' revertants was not observed both in the standard plate test and in the preincubation test either without S-9 mix or after the addition of a metabolizing system.

Conclusion: According to the results of the present study, the test substance Uvinul MS 40 is not mutagenic in the Ames test under the experimental conditions chosen.

(BASF AG 1990)

SCCS comment
According to OECD TG471, at least five strains of bacteria should be used. In the study, only four strains of Salmonella were used. No historical control data were provided.

Bacterial gene mutation test 3

Guideline:	OECD 471 Commission Regulation (EC) No 440/2008; B.13 / B.14 US EPA OPPTS 870.5100
Test system:	Escherichia coli reverse mutation assay (E. coli WP2 uvrA)
Replicates:	Triplicate plates, two independent assays
Test substance:	2-Hydroxy-4-methoxybenzophenon-5-sulfonic acid, CAS No: 4065-45-6
Batch:	Y7115C182
Purity:	98.8 g/100 g
Solvent:	Ultrapure water
Metabolic activ:	Phenobarbital i.p. and β-naphthoflavone orally induced rat liver S9 fraction
Positive controls:	4-nitroquinoline-N-oxide (4-NQO) without S9-Mix; Aminoanthracene(2-AA) with S9 mix
Concentrations:	0; 33; 100; 333; 1000; 2500 and 5000 µg/plate with and without metabolic activation
Treatment:	Standard plate test (SPT) and preincubation test (PIT) both with and without metabolic activation
GLP:	In compliance
Study period:	16 March – 12 May 2017

The test substance 2-Hydroxy-4-methoxybenzophenon-5-sulfonic acid was tested for its mutagenic potential based on the ability to induce point mutations in selected loci of Escherichia coli in a reverse mutation assay.

No bacteriotoxic effect (reduced trp- background growth, decrease in the number of trp+ revertants) was observed in the standard plate test up to the highest concentration.
In the preincubation assay bacteriotoxicity (reduced trp- background growth, decrease in the number of trp+ revertants) was observed depending on the test conditions from about 2500 µg/plate onward. No precipitation of the test substance was found with and without S9 mix. A bacteriotoxic effect was occasionally observed depending on the test conditions from about 2500 µg/plate onward.

Results: The test substance did not lead to a relevant increase in the number of revertant colonies without S9 mix or after adding a metabolizing system in two experiments carried out independently of each other (standard plate test and preincubation assay). Besides, the results of the negative as well as the positive controls performed in parallel corroborated the validity of this study, since the values fulfilled the acceptance criteria. In this study with and without S9 mix, the number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain. In addition, the positive control substances with and without S9 mix induced a significant increase in the number of revertant colonies within the range of the historical positive control data.

Conclusion: Under the experimental conditions of this study, the test substance 2-Hydroxy-4-methoxybenzophenon-5-sulfonic acid is not mutagenic in the Escherichia coli reverse mutation assay in the absence and the presence of metabolic activation.

(BASF AG 2017)

SCCS comment
The SCCS agrees that the results of this study are negative and benzophenone-4 is not mutagenic in the E.Coli WP2 uvrA strain.

***In vitro* mammalian chromosome aberration study**

Guideline:	No OECD TG is mentioned. Protocol E-437, ed. 3
Cells:	Chinese Hamster Ovary Cells (CHO-WBI)
Replicates:	Duplicate cultures in two independent experiments
Test substance:	2-Hydroxy-4-methoxy benzophenone-5-sulfonic acid, yellow/white powder
Batch:	Nr . 91-0835, ZSTNr. 88/52
Purity:	/
Solvent:	culture medium without serum
Metabolic activation:	Arochlor induced S9 fraction from rat liver
Positive controls:	Mitomycin-C (MMC: 500ng/ml and 1 µg/ml) for the nonactivation and Cyclophosphamide (CP: 25 and 50µg/ml) in the metabolic activation series
Concentrations:	In the presence and absence of S9-mix: 2.0, 3.0, 4.0 and 5 .0 mg/ml
Treatment:	Without S9-mix: 7.25 hours in the regular harvest and for 23.2 hours in the delayed harvest part then colcemid added With S9-mix: 2h then incubated 8.25h before adding colcemid
GLP:	In compliance
Study period:	February 15 – April 29, 1988

2-Hydroxy-4-methoxy benzophenone-5-sulfonic acid was investigated for clastogenic activity in the *in vitro* by measuring chromosomal aberration frequencies in Chinese hamster ovary cells. The assay was conducted using duplicate cultures in the presence and absence of a metabolic activation system.

The test article, was dissolved in culture medium. Solubility of the compound was evaluated in culture medium at and at concentration of 250.0 mg/ml a clear solution was obtained. A fifty-fold dilution of this stock solution into serum free culture medium resulted in a clear yellow solution: meaning that a distinct pH shift was caused by the test article. Sodium hydroxide was be used as the neutralizing agent. A series of lower doses in half log dilution steps were assayed with a lowest concentration of 166.7 µg/ml. Immediately after the addition of the test solution to the cultures the pH was adjusted to - 6.8 with 1N NaOH.

Range-finding test: Chinese hamster cell cultures (5 ml) were exposed to the test compound with and without S9-mix in a series of doses covering over four orders of magnitude with the top dose 5.0 mg/ml and the lower doses formed a half-log series.
In the range-finding assay with and without metabolic activation toxicity occurred at the two highest dose levels of 5.0 mg/ml and 1.7 mg/ml. Cell cycle kinetics were evaluated from 166.7 µg/ml through 5.0 mg/ml. For treatment without metabolic activation doses ranging from 0.1 mg/ml through 2.0 mg/ml were selected for the regular harvest part and concentrations of 3.0 mg/ml through 5.0 mg/ml were assayed in the delayed harvest part. A regular harvest time was chosen for the treatment with S9-mix with a dose range of 0.1 mg/ml through 5.0 mg/ml.

Chromosomal aberration test: Duplicate 10 ml cultures were used for each dose level, and for the two positive controls, single cultures were initiated for the untreated and solvent control (which were identical in this test). The positive controls were Mitomycin-C (MMC: 500ng/ml and 1 µg/ml) for the nonactivation and Cyclophosphamide (CP: 25 and 50µg/ml) in the metabolic activation series. To achieve the desired final concentrations of test article, 0.2 ml test solution was added per 10 ml culture.

Assay without metabolic activation system:
Exponentially growing cells were treated with the test article at 2.0, 3.0, 4.0 and 5.0 mg/ml doses for 7.25 hours in the regular harvest part and for 23.2 hours in the delayed harvest

part. The cultures were then washed and fresh culture medium was added with colcemid at a final concentration of 0.1 µg/ml.

Assay with the metabolic activation system:
Cells were treated with test article at 2.0, 3.0, 4.0 and 5.0 mg/ml in the presence of S9 mix in growth medium without fetal calf serum (FCS) for 2 hours, then washed and normal growth medium containing 10**%** FCS was added. Incubation was continued for a further 8.25 hours. Colcemid was present during the last 2.7 hours of incubation.

Metaphase cells were then harvested and prepared for cytogenetic analysis. One hundred cells from each duplicate culture for at least four dose levels of the test article and from each of the untreated and solvent control cultures were analyzed for chromosomal aberrations.

Results: There was a normal background level of aberrations in the negative controls. Both positive controls induce significant level of chromosomal damage. Cytotoxicity was observed at 5.0mg/ml both with and without metabolic activation. No statistically significant increases in aberrations was observed in any concentration tested both with and without metabolic activation. Therefore, the test article was considered negative.

Conclusion: The test article, 2-Hydroxy-4-methoxy benzophenone-5-sulfonic acid, Batch Nr. 91-0835, ZST Nr . 88/52, was considered negative for chromosomal aberration induction in Chinese hamster ovary cells both with and without metabolic activation S9-mix.

(Hazleton Biotechnologies 1988)

SCCS comment
The SCCS agrees with the conclusion of the applicant that benzopenone-4 does not induce chromosomal damage under the study conditions. No information on batch Nr. 91-0835 (impurities, etc.) is included in the study report. No OECD TG is mentioned. Historical controls were provided. Only 200 metaphases were analysed (instead of the presently recommended 300).

***In vitro* mammalian cell gene mutation assay**

Guideline:	OECD TG 476 (2016)
Cells:	Chinese Hamster Ovary Cells CHO cells [*Hprt* locus for 6-thioguanine (6-TG) resistance]
Replicates:	Single experiment, Triplicate culture
Test substance:	Sulisobenzone [CAS No.: 4065-45-6), EC 223-772-2, Lot BP4-l 012004/21
Batch:	SB00l
Purity:	Relative purity 99.7%
Solvent:	Distilled water
Metabolic activation:	Phenobarbital (PB) and β-naphthoflavone (BNF) induced rat liver
Positive controls:	Ethyl methanesulfonate (EMS) 400ug/ml, Benzo(a)pyrene 30ug/ml
Concentrations:	0.25, 0.5, 1, 2 mg/ml with and without metabolic activation
Treatment:	4h without and with S9-mix
Expression period:	7 days
GLP:	In compliance
Study period:	August 5 – October 10, 2022

Sulisobenzone was tested for mutagenic effects in Chinese hamster ovary cells at 0 (solvent control), 0.25, 0.5, 1, 2 mg/ml with and without metabolic activation. Based on the solubility and precipitation test, a preliminary cytotoxicity testing was performed with the Test Item at the following concentrations: 0.125, 0.25, 0.5, 1 and 2 mg/ml in the culture medium, both in the presence and absence of metabolic activation system along with vehicle (distilled water) control.

The relative survival value for the highest concentration 2mg/ml was in the absence of metabolic activation 59.27% and in the presence of metabolic activation 56.52%. As no precipitate and limiting cytotoxicity (RS: 10-20%) were observed, 2 mg/ml was selected as the highest test concentration, which is also recommended by the test guideline.
In the main study, cultures were exposed to vehicle control, different concentrations of the Test Item, and positive control for 4 hours (short-term exposure) in the absence and presence of metabolic activation.

Results: No significant increase in the mutation frequency (MF) either in absence or presence of metabolic activation was observed when compared to vehicle controls.
The positive controls (Ethylmethanesulfonate and Benzo[a]pyrene) produced statistically significant increases in mutation frequency indicating the sensitivity of the test system to specific mutagens, confirmed that the test conditions were appropriate and that the metabolic activation system functioned properly. The results indicated that the Test Item, i.e. Sulisobenzone [CAS No.: 4065-4 5-6], did not induce a statistically significant or biologically relevant increase in the mutation frequency at concentrations of 0.25, 0.5, 1 and 2 mg/ml when compared to the vehicle control either in the presence or in the absence of S9 metabolic activation.

Conclusion: Based on the results of this study, it is concluded that Sulisobenzone [CAS No.: 4065-45-6] does not induce gene mutation at the locus of hypoxanthine-guanine phosphoribosyltransferase (Hprt) in CHO cells up to the concentration of 2 mg/ml of culture medium, either in the presence or absence of S9 metabolic activation system, under the experimental conditions described.

(Diligence Bio Pvt. Ltd. 2022)

SCCS comment
Although benzophenone-4 was studied at each concentration only in single culture, the SCCS agrees with the study results indicating that benzophenone-4 is negative in mammalian gene mutation tests.

Benzophenone-4 in water as solvent, was tested according to OECD Test Guideline 476 assay for mutagenic effects in Chinese hamster ovary cells at 0 (solvent control), 1, 2.5, 5 and 10 mM with and without metabolic activation. The study was performed under GLP conditions. 7,12-dimethylbenz(a) anthracene and N-ethyl-N-nitrosourea were used as positive controls with and without metabolic activation respectively. Treatment with N-ethyl-N-nitrosourea produced a significant increase in the number of revertant colonies whereas 7,12-dimethylbenz(a) anthracene did not. Without metabolic activation, benzophenone-4 did not produce a significant increase in the number of revertant colonies when compared to control data. No conclusion could be drawn regarding the mutagenicity of the test chemical in CHO cells in the presence of metabolic activation.

(unnamed study report 2015 from EU REACH record)

SCCS comment
This study is not valid, as the positive control did not fulfil the necessary criteria.

Conclusion by the applicant: With the evidence from an *in vitro* battery of tests, benzophenone-4 is considered non-mutagenic.

SCCS overall conclusion on mutagenicity / genotoxicity *in vitro*
Benzophenone-4 was tested for mutagenic/genotoxic potential in an adequate battery of *in vitro* tests with various genotoxicity endpoints. Three studies on potential mutagenicity by the Ames test at concentrations up to 5000 µg/plate with and without metabolic activation

were negative. The valid Mammalian gene mutation test in CHO cells was also negative. The *in vitro* chromosome aberration test in CHO cells was negative.

Based on the present reports, the SCCS considers benzophenone-4 to have no *in vitro* genotoxicity potential.

3.4.6.2 Mutagenicity / genotoxicity *in vivo*

No *in vivo* data were submitted.

3.4.7 Carcinogenicity

/

3.4.8 Photo-induced toxicity

3.4.8.1 Phototoxicity / photo-irritation and photosensitisation

Animal *in vivo* data:

Guideline:	/
Test material:	Benzophenone-4
Vehicle:	40% dimethylacetamide, 330% acetone and 30% ethanol (DAE)
Purity:	98.3%
Species/strain:	Guinea pigs / Dunkin-Hartley
No. of animals:	Photo irritation: 5 animals each per control and treated groups Photosensitization: 10 animals each per control and treated groups and additionally, a vehicle control group with 5 animals
Irradiation:	UV-A = 10 Joules/cm^2 and UV-B = 0.1 Joules/cm^2
Positive control:	Photo irritation: 8-methoxypsoralen Photo sensitization: 3,5,4 – tribromosalicylanilide Photo-Irritation: 0, 1, 10 and 50% w/v Photosensitization: Intradermal: FCA at a 50% v/v Topical:5% and 10% w/v Challenge: 5% and 10% w/v
Year:	1996

In a study using a guinea pig model, the test substance benzophenone-4 was evaluated for its potential to cause photo irritation and photosensitization. The study consisted of two main phases. In the first phase of the study, different concentrations of the test substance (50%, 10%, and 1% w/v) were applied to groups of five animals with and without exposure to UV light to assess its photo-irritant effects. No irritation was observed with the test substance, while a reference substance called 8-methoxypsoralen showed marked photo-irritation. In the second phase, the photo sensitizing potential of the test substance was investigated using three groups of ten animals each. Animals were induced with the test substance by intradermal injection followed by topical application and exposure to UV-A and UV-B light. A positive control group of ten animals was induced with a substance (3,5,4-tribromosalicylanilide), and a vehicle control group of five animals was treated with the vehicle alone. After a two-week period, the animals were challenged with the test substance and the vehicle, followed by exposure to UV light. A minor response was noted in some animals at the sites challenged with the vehicle, which was reversible. However, a high incidence of reaction to the test substance was observed in animals induced with the 10% concentration, both with

and without UV light exposure. The group induced with the substance but not exposed to UV light showed a similar response. Animals induced and challenged with a lower concentration of 5% showed a reduced response compared to the 10% concentration group. The positive control reference substance successfully demonstrated photosensitization in the animals. Based on the results, it can be concluded that the test substance at a 10% concentration acts as a sensitizer in the guinea pig model. The response was not photo-induced, as there was no significant difference between the groups exposed to UV light and those without UV light exposure. Additionally, the lack of difference in response between irradiated and non-irradiated sites during the challenge suggests that the reaction was not photo mediated. The reduced response at a lower concentration of 5% indicates a dose-response relationship and the possibility of a threshold concentration required to induce sensitization.

(Nunziata 1996)

SCCS comment

The SCCS confirms the SCCNFP conclusion that benzophenone-4 is not phototoxic in guinea pigs.

Human *in vivo* data:

Benzophenone-4, ranging from 1% to 10% concentration, have undergone extensive testing on over 167 individuals to evaluate their effectiveness as sunscreens. These tests were conducted using different sources of UV radiation, such as solar simulators, sunlight, ultraviolet lamps, hot quartz lamps, xenon arc solar simulation, germicidal mercury lamps, and quartz mercury lamps. Across all the tests conducted, no instances of irritation or phototoxic reactions were reported in relation to these ingredients.

(CIR 1983)

In a study by Manciet *et al*. (1994), 144 patients with dermatoses were examined for photosensitivity. Two cases of contact reactions caused by benzophenone-4 were found: one was allergic and the other was a photoallergic reaction with UVB. Photopatch studies showed that seven and two patients with photodermatitis from benzophenone based antirheumatics did not exhibit cross-reactivity to benzophenone-4.

Fisher's findings, based on patch testing hundreds of patients over ten years, revealed only two cases of allergy to benzophenone-4. He concluded that this indicated a very low sensitivity in the population and deemed benzophenone-4 safe for general use. Fotiades *et al.* (1995) mentioned that the North American Contact Dermatitis Group considers benzophenone-4 a photopatch allergen, but they provided no further evidence.

In conclusion, the limited number of reported cases in the literature compared to the potential exposure of many individuals over the past 30 years suggests that benzophenone-4 either has no clinically significant sensitizing potential or a very low, almost negligible, potential when used in sunscreen concentrations up to 10%.

(SCCNFP/0085/98)

SCCS conclusion

The SCCS confirms the SCCNFP conclusion that benzophenone-4 is not phototoxic in an *in vivo* guinea pig study. Based on human data there is low evidence for phototoxicity.

3.4.8.2 Photomutagenicity / photoclastogenicity

Chromosomal aberration test in cultured Chinese hamster ovary (CHO cells in the presence of UV light

Guideline:	No OECD TG is mentioned
Cells:	Chinese Hamster Ovary Cells (CHOI)
Replicates:	Duplicate cultures in two independent experiments
Test substance:	2-Hydroxy-4-methoxy benzophenone-5-sulfonic acid (sulisobenzone), yellow/white powder
Batch:	2404202, ZHT No 95/72
Purity:	98.3 g/100 g. It contains 0 .3 g/100 g sulfuric acid and 0.2 g/100 g of a chemically related by-product
Solvent:	Sterile ROHP water
Activation:	UV light 1500 and 750 mJ/cm^2
Negative control:	Solvent control, 8-methoxypsoralen (8-MOP) in the absence of UV light
Positive controls:	4-Nitroquinoline-l-oxide in the absence of UV light, 8-methoxypsoralen (8-MOP) in the presence of UV light
Concentrations:	1511, 2159 and 3084 µg/ml in the presence and absence of UV light (at 1500 mJ/cm^2)
Treatment:	3 hours with or without irradiation and 17h recovery
GLP:	In compliance
Study period:	May 16 1995 – January 11, 1996

2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (sulisobenzone) was tested in an *in vitro* cytogenetics assay using duplicate cultures of CHO cells in the presence and absence of UV light.
A preliminary range-finder, covering a broad range of doses, was performed in the presence of two doses of UV radiation to investigate the phototoxicity of the chemical, and to determine the dose range to be used in the main study. The highest dose level used in the range-finder, 3083 µg/mL, was a concentration of 10 mM in culture medium. The doses of UV used were 1500 and 750 mJ/cm^2. In the phototoxicity range-finder, there was no marked mitotic inhibition shown following irradiation at either of the two levels (1500 and 750 mJ/cm^2), and only the higher UV dose was used in the main study. The concentration of 3084 µg/mL (approximately 10 mM) was chosen as the top dose for the main study and a range of doses from this concentration were used in the absence and presence of UV radiation.

The test article dose levels for chromosome analysis from the irradiated cultures were selected by evaluating the effect of 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (sulisobenzone) on mitotic index. Chromosome aberrations were analysed at 3 consecutive dose levels 1511, 2159 and 3084 µg/ml in the presence and absence of UV light (at 1500 mJ/cm^2). The highest concentration chosen for analysis, 3084 µg/mL, did not induce mitotic inhibition in either the absence or presence of UV radiation.

Appropriate negative (solvent) control cultures were included in the test system in both experiments under each treatment condition. The proportion of cells with structural aberrations in these cultures fell within historical solvent control ranges. 4-Nitroquinoline-l-oxide and 8-methoxypsoralen (8-MOP) were employed as positive control chemicals in the absence and presence of UV radiation, respectively.
Both treatments induced increases in the proportion of cells with structural aberrations. When added to cultures treated in the absence of UV radiation, 8-MOP induced frequencies of cells with structural aberrations that were similar to those seen in concurrent solvent control cultures (non irradiated).

Treatment of cultures with 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (sulisobenzone) in the absence of UV radiationresulted in frequencies of cells with structural aberrations that were similar to and not significantly different from the frequency seen in concurrent, non irradiated, solvent control cultures. Aberrant cell frequency was within the historical negative control range.

Treatment of cultures with 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (sulisobenzone) in the presence of UV radiation resulted in frequencies of cells with structural aberrations that were not increased compared to the concurrent, irradiated, solvent control cultures. The frequency of cells with structural aberrations was, in fact, reduced below the frequency seen in the concurrent, irradiated, solvent control cultures at all three concentrations of 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (sulisobenzone) analysed. This apparent protective effect reduced as the concentration of 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (sulisobenzone) increased, and the reason for this is not clear.

It is concluded that 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (sulisobenzone) did not induce chromosome aberrations in cultured CHO cells in the absence or presence of UV radiation.

SCCS comment

The highest concentration induced significantly higher numerical chromosomal aberrations in the presence of UV radiation. UV significantly induced structural chromosomal aberrations in CHO cells. However, positive control 8-methoxypsoralen did not induce significantly higher frequency of chromosomal aberrations in the presence of UV radiation compared to UV radiation alone. Only 200 metaphases were analysed per sample (instead of the presently recommended 300). In positive controls, only a small number of cells (75 or 50, resp.) were analysed. No historical positive controls data are provided.

Another study involved assessing the test organism E. coli WP2 for tryptophan dependence and the absence of pKM101 (ampicillin resistance) in accordance with GLP guidelines from the UK and OECD. The active ingredient was dissolved in purified and filtered water, while positive control substances, 4-nitroquinoline-N-oxide (without ultraviolet radiation) and 8-methoxypsoralen (with ultraviolet radiation), were dissolved in DMSO. Ultraviolet radiation, produced by a "Heraeus suntest" lamp, was used with two levels (UVA and UVB), and its intensity was measured with an Osram "Centra" UV meter. The tests were performed in triplicate. In the initial range finder test, 5 to 5000 µg/plate concentrations of the active ingredient were used, revealing toxicity at the highest concentrations. Therefore, definitive tests were conducted using 200 µg/plate as the primary concentration and its half-log diminutions: 200, 63.25, 20, and 6.325 µg/plate. The level of 200 µg/plate in the presence of ultraviolet radiation was established as the lower limit of toxicity. Notably, no photomutagenic activity was observed during the experiments. The positive controls exhibited significant increases in revertants, confirming their effectiveness in the study. However, the test with the active ingredient yielded negative results, indicating no mutagenic effects.

(Ballantyne *et al.* 1996 cited in SCCNFP/0085/98)

SCCS overall conclusion on photomutagenicity / photoclastogenicity

The SCCS confirms the SCCNFP conclusion that benzophenone-4 is unlikely to pose a photomutagenic or photoclastogenic risk under the specific test conditions employed.

3.4.9 Human data

Phototoxicity data:

In several studie, the phototoxicity of cosmetic products containing benzophenone-4 (at concentrations ranging from 0.1% to 3.5%) was assessed using human subjects (CIR 1983). A small number of studies reported instances of photosensitization caused by benzophenone-4, although the occurrence was relatively low.

Table 6: Phototoxicity studies for benzophenone-4 dosed in human studies.

Method details	Observations	Reference
10% in petrolatum. 402 human subjects with suspected clinical photosensitivity. Patch and photopatch tests performed according to ICDRG guidelines. UVA dose of 5 or 10 J/cm^2 used for photopatch testing.	No photoallergic or allergic reactions	Schauder & Ippen 1997
5% and 10% in white paraffin. 1155 human subjects (from 17 centres across the UK, Ireland and the Netherlands). Photopatch testing (over 2-year period) involved application of test substance (on aluminium Finn chamber) to skin of mid-upper back (paravertebral area avoided) for 24 hours or 48 hours (depending on the centre). Contact dermatitis units traditionally applied allergens for 48 hours, and photobiology units traditionally applied allergens for 24 hours. Following patch removal, one set (dark control) covered with light-impermeable occlusive dressing, and the other set irradiated with fluorescent UVA (5 J/cm^2). Reactions scored at 48 hours post-irradiation, and, if possible, at 24 hours and 72 hours. ICDRG visual scoring system was used.	Photoallergy in 2 and 5 patients, in 5 and 10% dose groups, respectively. Contact allergy in 2 and 9 patients, in 5 and 10% dose groups, respectively. Irritation reactions observed in 2 and 4 patients, in 5 and 10% dose groups, respectively.	Schauder & Ippen 1997
Not stated. 12 human subjects with history of acute eruption on photoexposed areas (induced by ketoprofen or tiaprofenic acid). At least 1 month after acute episode of contact dermatitis, patients patch tested using Finn chamber technique. Finn chambers mounted on Scanpor tape, and patches removed after 2 days. For UV irradiation, 2 sources of light used (UVA alone and UVA + UVB).	Photopatch test results negative for Benzophenone-4.	Le Coz *et al.* 1998
Not stated. 82 human subjects (with clinical diagnosis of photoallergic contact dermatitis). Study performed to identify photoallergens that caused photoallergic contact dermatitis in population attending outpatient clinic in Columbia. Test substances applied, in duplicate, to skin on back. Test sites covered with opaque tape for 24 hours. Panel on right irradiated with UVA (dose = 5 J/cm^2; irradiance = 10.4 mW/cm^2). Reactions scored 24 hours after application and at 24 hours and 72 hours post-irradiation.	Benzophenone-4 induced a positive photopatch reaction in 2 of 82 patients (2.4%).	Rodriguez *et. al* 2006

Not stated. 160 human subjects (37 male, 123 female). Retrospective chart review on patients who underwent photopatch testing in Canada between January of 2001 and December of 2010. Duplicate sets of allergens applied to back. At 24 hours, 1 set of allergens uncovered and exposed to UVA at a dose of 5 J/cm^2. Other set of allergens shielded from UVA exposure. 24-hour reactions to non- irradiated compounds assessed at 15 to 20 minutes later. On following day, irradiated patches read at 24 hours post- irradiation. Reactions at non-irradiated patch test sites read 48 hours after application.	Benzophenone-4 caused allergic contact dermatitis in 3 patients, but did not cause photoallergic reactions.	Rodriguez *et al.* 2006
10% (in petrolatum). 15 eczematous dermatitis human subjects. Photoallergenicity testing performed at least 3 months after complete disappearance of the dermatitis. In photopatch tests, test substance applied to back, under occlusion, over 2-day period. At 24 hours, occlusive patch removed and site exposed to UVA (5 J/cm^2). Reactions scored at 48 hours and 96 hours (day 2 and day 4).	No positive reactions to Benzophenone- 4.	Foti *et al.* 2008
Not stated. 7 human subjects with ketoprofen-induced photodermatitis. Study performed to evaluate possibility of cross-reactivity between ketoprofen and benzophenones and other chemicals. Patch tests (uninvolved skin of back) performed using Finn chambers. At 24 hours post- application, separate series of patch tests exposed to suberythemal doses of UVB and UVA. Irradiated and non-irradiated sites evaluated at 72 hours post-application.	One subject had weak positive reaction (+ reaction), with no concomitant erythema score, at irradiated site.	Kerr *et al.* 2009
2% in petrolatum. 347 human subjects from 12 European countries. Test substance applied to back skin, and removed at 48 hours. One site irradiated with UVA (5J/cm^2) and other site covered with UV-impermeable material. Reactions were scored at 48 hours.	Photoallergy elicited in 3 patients (+reaction (1 patient) and ++ reaction (2 patients)	EMCPPTS 2012
2% in petrolatum. 1000 human subjects (consecutive dermatology outpatients in Poland). In study group, 36 (3.6%; 95% CI: 2.4-4.8%) individuals required photopatch testing based on their clinical symptoms. Because total number of patients requiring patch tests of any kind amounted to 205, percentage of photopatch tested patients among all patch-tested patients was 17.5% (95% CI: 12.2-22.8%). Patch tests (2 identical sets) mounted on back and remained under occlusion for 48 hours. Some sites irradiated with UVA (5 J/cm^2) and some non-irradiated. Skin reactions scored 24 hours and 48 hours after irradiation. Presence of inflammatory reaction at irradiated sites and no reaction to same hapten at non- irradiated sites interpreted as confirmation of photoallergy. In case of positive reactions to a hapten, at both irradiated and non-irradiated sites, the classical contact allergy was recognised.	One patient had a positive photoallergy reaction	Spiewak 2013

Not stated. 157 children (69 male, 88 female). Duplicate series of UV filters and children's own sunscreen products applied to back. Reactions scored at time of sample removal and at 24 hours and 48 hours after exposure to UVA (5 J/cm^2).	Single case of photoaugmentation reaction to Benzophenone-4 reported. Patient had + reaction in control panel, but had ++ reaction in irradiated panel	Greenspoon *et al*. 2013

SCCS comment

The phototoxicity studies on human subjects mentioned by the applicant were reviewed, but were of limited value for the current assessment due to:

- lack of clarity regarding the test substance, making it difficult to determine whether the test substance was based on the product or the individual ingredient
- undefined concentration of the ingredient
- absence of method description
- no full study reports.

Nevertheless, the risk for phototoxicity in humans is very low.

Allergic patch testing data:

See Table 4 section 3.4.2.

Nevertheless, the risk for photosensitivity in humans is very low.

3.4.10 Special investigations

3.4.10.1 Non-test information, *in silico*, read across, *in chemico*

/

3.4.10.2 *In vitro* assays

Research investigating the effects of benzophenone-4 on thyroid hormones T3 and T4 was conducted using GH3 cells and FRTL-5 cells. In GH3 cells, a test concentration of up to 98.7 mg/l (320 µM) of benzophenone-4 did not affect the regulation of Tshβ and Trhr genes but led to the downregulation of the Trβ gene. On the other hand, in FRTL-5 cells, benzophenone-4 (at concentrations ranging from 10 to 320 µM) significantly upregulated the Nis and Tg genes while down-regulating the Tpo gene. Furthermore, zebrafish larvae treated with benzophenone-4 exhibited a statistically significant decrease in triiodothyronine (T3) levels at a test concentration of 32 µg/l, but no significant change was observed in thyroxine (T4) levels (CIR 2021).

Benzophenone-4 underwent testing as part of the US EPA ToxCast program, where it demonstrated weak active responses in only 3 out of 430 assays. The response in the estrogen response element (ERE) assay and the retinoid X receptor (RXR) assay was just slightly above the cutoff data. Although there was a weak positive response in the androgen receptor (AR) agonist assay, the data was deemed to be very noisy. As a result, benzophenone-4 was considered negative in both the ToxCast AR and ER pathways models. The overall very low bioactivity observed in the ToxCast assays suggests that follow-up toxicity testing is of low priority for this compound (Judson *et al.* 2015, 2020).

In the US EPA Endocrine Disruption Screening Program (EDSP) report (accessible at https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID2042436), benzophenone-4 underwent testing in 18 estrogen receptor assays, and the results showed

only one weak positive observation. However, there was no activity detected in 14 androgen receptor assays, 9 thyroid receptor assays, or 2 steroidogenesis assays.

Other *in vitro* studies are summarised in Table 7.

Table 7:

Method details	Observations	Reference
Toxicity to reproduction: other studies no guideline followed The activity of various phenolic additives, including Benzophenone-4, were assessed in a recombinant yeast estrogen assay. In brief, yeast cells transfected with the human estrogen receptor α(ERα) gene, togehter with expression plasmids (containing estrogen responsive elements and the lac-Z reporter gene encoding the enzyme β-galactosidase), were incubated in medium containing the test chemical and the chromogenic substrate, chlorophenol red-β-D-galactopyranoside (CPRG). Active ligands (which bind to the receptor) induce β-galactosidase (β- gal) expression, and this causes the CPRG (initially yellow) to change into a red product that can be measured by absorbance. Absorbance readings were made at 540 nm using a Spectramax 340 PC plate reader (Molecular Devices, Sunnyvale, CA).	The test item displayed no detectable estrogenic activity.	Miller *et al.* 2001
Toxicity to reproduction: other studies according to guideline Nishikawa J., Goto J., Saito K., Matsuo M. and Nishihara T. (1998): Use of yeast two hybrid system to analyze environmental estrogen. Jpn. J. Toxicol. Environ. Health, 44, P-32. In this study, 19 hydroxylated benzophenones, including the test substance Benzophenone-4, were tested for estrogenic activity by a yeast two- hybrid assay. This system is based on the ligand-dependent interaction of estrogen receptor (ER) α and the coactivator transcriptional intermediary factor 2 (TIF2), and the estrogenic activity was detected as β-galactosidase activity. Two expression plasmids, pGBT9-estrogen receptor ligand domain (pGBT9 -ERLBD) and pGAD424 -TIF2, were introduced into yeast cells (Saccaromyces cervisiae Y190), which carry a β-galactosidase reporter gene and require tryptophan and leucine for growth. The test	Benzophenone-4 showed no estrogenic activity in the yeast two-hybrid assay (REC10 > 1.0 x 10^-3 M).	Kawamura *et al*. 2003

chemicals were dissolved in dimethylsulfoxide (DMSO; 1%). Each experiment was accompanied by E2 (17β-estradiol) as a positive control and DMSO as a negative control. The results were evaluated based on relative activity, expressed as 10% relative effective concentration (REC10), which is the concentration of the test chemical showing 10% of the agonist activity of 10^-6 M E2, the highest activity level of E2. When the activity of the test chemical was higher than the EC10 within the concentration tested, the chemical was judged to be positive.		
Toxicity to reproduction: other studies according to guideline to the protocol of Wilson *et al*. (2002) with several modifications. In this study the potential agonistic and antagonistic actions of various UV filters, including Benzophenone-4, on androgen receptors (AR) in the human breast carcinoma cell line MDA-kb2, were assessed. The MDA-kb2 cell line expresses high levels of functional endogenous androgen receptor (AR) and also glucocorticoid receptor (GR) and is stably transfected with luciferase transporter plasmid driven by the mouse mammary tumor virus promoter (MMTV) that can be activated through both AR and GR. Compounds activity through AR or GR can therefore induce luciferase expression. In this study the cells were exposed to different concentrations of the test item in ethanol, in the absence or presence of 0.1 or 0.5 nM Dihydrotestosterone (DHT), a known androgenic chemical. Cells were incubated over night at 37 °C. Afterwards the luciferase activity was measured.	The test item displayed no agonistic activity.	Ma *et al.* 2003
Toxicity to reproduction: other studies no guideline followed Estrogenic and antiandrogenic activities of various benzophenone derivatives, including Benzophenone-4, were comparatively examined with hormone- responsive reporter assay in various cell lines. Estrogenic activity of the test substance was examined using ERE- luciferase reporter assay in MCF-7 cells. Androgenic activity of the test substance was examined using NIH3T3 cells transfected with a luciferase receptor gene.	Antiandrogenic IC50: >100 µM based on: (test mat.) Estrogenic EC50: >100 µM based on: (test mat.) The test substance was negative in the estrogen screening assay. Dibydrotestosterone (DHT) exhibited marked androgenic activity toward NIH3T3 cells at 1 x 10 ^-11 to 1 x 10^-8 M. No androgen activity of the test item alone was observed in a concentration range of 10^-7 to 10^-4 M. When the test item was added to the DHT assay system in the concentration range of 1 x 10^-8 to 1	Suzuki *et al*. 2005

	x 10^- 5 M, no significant inhibitory effects on the androgenic activity of DHT in rat fibroblast cell line NIH3T3 was shown. IC50 value of the test substance against the androgenic activity of 1 x 10^-10 M DHT is >100 µM. Accordingly, the test substance showed very low estrogenic and antiandrogenic activity in the present study.	
Toxicity to reproduction: other studies no guideline followed Benzophenone-4 was evaluated for estrogen agonistic and/or antagonistic activity using two *in vitro* assays, (1) an ELISA-based estrogen receptor competitive binding assay (ER-ELISA) and (2) a modified yeast two-hybrid estrogen assay, with and without addition of a rat liver preparation, S9 mix. Diethylstilbestrol (DES) and 17β-estradiol (E2) were used as positive controls.	The test item was negative in the ER-ELISA assay with and without S9 mix (relative binding affinity to DES < 0.022). No agonistic (relative activity to E2 < 0.0013) or antagonistic effects (EC50 > 5000 nM) were observed in the two-hybrid assay with and without S9 mix.	Morohoshi *et al.* 2005
Toxicity to reproduction: other studies no guideline followed In this study, the estrogenic and antiandrogenic activities of various Benzophenones, including Benzophenone-4, were measured using transfected human estrogen and androgen receptors in Chinese hamster ovary cells. The estrogenic activity of the test substance was investigated using ERα- EcoScreen™. For the evaluation of the results, the 50% effective concentration (EC50) value and the 50 % positive concentration (PC50) value were calculated. The EC50 value was the concentration of the test chemical corresponding to 50 % of its maximum luciferase activity and the PC50 value was that corresponding to 50% of the maximum luciferase activity of the positive control (10^-9 M E2). The AR agonist activity of the test substance was examined using the AR– EcoScreen™cell line. The anti-androgenic activity of the test item was evaluated using the AR- EcoScreen™ in the presence of 5 x 10^- 10 M DHT. For the determination of the anti-androgenic activity, the IC50 was calculated. The 50% inhibition concentration (IC50) value was the concentration of the test chemical corresponding to 50% inhibition of 5 x 10^-10 M DHT-induced luciferase activity.	In this experiment, the test item displayed no estrogenic activity (EC50/PC50 > 1 x 10^-4 M). No androgenic activity of the test substance was determined (EC50 > 1 x 10^-4 M). An anti-androgenic effect has not been demonstrated in this experiment for the test substance(IC50 > 1 x 10^-4 M).	Kawamura *et al.* 2005

Toxicity to reproduction: other studies no guideline followed In this study a simple detection system in Escherichia coli was used to detect estrogenic activity of different consumer products. The engineered E. coli biosensor strain expresses the ligand-binding domain of the human estrogen receptor β (ERβ) as part of a larger allosteric reporter enzyme. Expression of the reporter protein creates a hormone dependent growth phenotype in thymidylate synthase deficient E. coli strains. Thus, these strains report estrogenicity via changes in growth rate on knockout medium, as detected by a simple measure of the turbidity of the liquid culture in the presence of various test compounds. In this assay, this system was employed to assay for estrogenic behavior in complex mixtures found in consumer products. All experiments were carried out in E. coli strain D1210 harboring the pMIT::ERβ biosensor plasmid or pMIT:: TR* control plasmid. A total of five cosmetic washing products and one perfume were examined, in which Benzophenone-4 is the suspected estrogenic ingredient. 17-β-estradiol, a known estrogen, and two non- interacting thyroid hormones served as controls.	As expected, growth of the pMIT::ERβ strain greatly increased in the presence of 17-β-estradiol, while the pMIT::TR* strain was unaffected. However, in the presence of the thyroid-hormones 3,3',5-triiodothyroacetic acid (Triac) and 3,3',5- triiodo-L-thyronine (T3), the pMIT::ERβ strain did not grow more than the negative control, while the pMIT::TR* strain showed greatly increased growth. As expected, in the presence of 50 µg/mL of thymine, which eliminates the selectivity of the growth medium, both strains grew to saturation within 24 h. These results confirm the estrogenic responsiveness of the pMIT::ERβ strain. The tested cosmetic washing products and the perfume, in which Benzophenone-4 is the suspected estrogenic ingredient, all showed a decrease in growth in both pMIT::ERβ and pMIT::TR* strains. The results suggest bacterial cell toxicity of the test substance. This is consistent with the properties of cleaning products. The results do not allow conclusions about an estrogenic activity of the test substance.	Gawrys *et al*. 2009
Toxicity to reproduction: other studies no guideline followed In this publication, data from studies on transcriptional activation (TA) testing with fish and human ERs were collected. TA assays are considered to identify chemicals that have the potential to transactivate ERs and are able to distinguish both estrogenic and anti- estrogenic properties of chemicals. The result in the literature have been reported as fold induction of enzyme activity produced by the test chemical relative to the untreated controls or the concentration at which 50% of the maximum induction was reached (EC50). Benzophenone-4 was investigated among other chemicals. The test substance was tested in three different TA assays. The estrogenic activity of the test substance was tested in an TA assay with human hERα in human breast cancer cell line MCF-7.	E(I)C50: 289 µM based on: (test mat.) Type of ERs: rtERα; Cells: yeast (Reference. Kunz *et al*. 2006) E(I)C50: 94.8 µM based on: (test mat.) Type of ERs: hERα, Cells: yeast (Reference: Kunz *et al.* 2006) An estrogenic activity was partially observed but the EC50 values are also very low compared to substances with known estrogenic activity (e.g. 17β-Estradiol). For this reason, the result is considered negative. E(I)C50: >100 µM based on: (test mat.) Type of ERs: hER; Cells: MCF-7 (Reference: Suzuki *et al.* 2005) Based on the result of this study, an estrogenic activity of the test substance is not expected.	Dang *et al*. 2011

In addition, the estrogenic activity of the test substance was determined employing a recombinant yeast carrying the estrogen receptor of rainbow trout (rtERα) and yeast carrying the human hERα.		

3.4.10.3 *In vivo* assays that provide data about selected endocrine mechanism(s) / pathway(s)

Hershberger assay (OECD TG441):

The anti-androgen potential of benzophenone-4 was investigated in a Hershberger assay in Wistar rats. Benzophenone-4 was administered to groups of 6 castrated but with testosterone propionate (TP; 0.4 mg/kg) treated (castrated animals substituted with testosterone); male Wistar rats by gavage for 10 days at dose levels of 300 and 1000 mg/kg bw/day. A vehicle control group was treated with corn oil by gavage and another control group was administered subcutaneously 0.4 mg/kg bw/day testosterone propionate to test the androgenic efficacy. Food consumption and body weights were determined and animals were examined for signs of toxicity or mortality. Clinicochemical examinations (incl. hormonal measurements, i.e. testosterone, dihydrotestosterone and luteinizing hormone) were performed towards the end of the administration period. Finally, all animals were assessed by gross pathology, followed by histopathological evaluations. During clinical examinations no benzophenone-4 related findings were observed. Concerning hormone investigations, co-administration of 1000 mg/kg bw/day benzophenone-4 and 0.4 mg/kg bw/day of testosterone propionate did not affect the hormone levels of testosterone and dihydrotestosterone in the serum of castrated male rats when compared with castrated animals given 0.4 mg/kg bw/day of testosterone propionate as reference androgen, only. A significant decrease in luteinizing hormone levels in the serum of benzophenone-4 treated rats was considered by the authors to be an incidental finding. Regarding pathology, the anti-androgen potential of a chemical is expressed in a deficient maturation of the accessory sex organs compared with the corresponding control. As expected, in castrated animals the absolute and relative weights of the accessory sex organs were significantly reduced when compared to castrated animals treated with testosterone propionate, only. Histologically, prostate, seminal vesicle and bulbo-urethral gland were immature. After treatment with benzophenone-4 in the presence of testosterone propionate, the absolute and relative weights of the accessory sex organs were not significantly changed. Moreover, the histology of prostate, seminal vesicle and bulbo-urethral gland was comparable to the respective TP control group. In conclusion, under the conditions of the present study, regarding clinical examinations, hormone investigations as well as pathological evaluations, no indications for an antiandrogen potential of benzophenone-4 were determined.

(BASF AG 2003)

Uterotrophic assay (OECD TG 440):

In an uterotrophic assay, benzophenone-4 (500 and 1000 mg/kg bw/d) was administered to 10 immature Wistar female rats (22 days of age at the beginning of the administration period) per group for 4 consecutive days twice daily by gavage. The aim of this study was to investigate the ability of benzophenone-4 with regard to a possible estrogenic (uterotrophic) effect on the absolute and relative uterine weights. Control groups consisted of vehicle (negative control) and diethylstilbestrol-dipropionate (positive control) treated rats. The administration of benzophenone-4 in both dose groups (500, 1000 mg/kg bw/d) elicited statistically significantly decreased body weight gains at the end of the administration period. In addition, in the 1000 mg/kg dose group body weight gain was also statistically significantly reduced over the entire study period. However, no clinical findings were recorded in the course of the study. No substance-related effects on the absolute and relative uterine weights were observed in the benzophenone-4 treated groups. Administration of 5 µg DES-DP/kg bw/d - a known uterotrophic agent - resulted in an approximate 3.7-fold increase in absolute and in

relative uterine weights in comparison to the vehicle control group values. Thus, under the conditions of this uterotrophic assay, benzophenone-4 did not promote growth of the uterus and is concluded therefore, not to have exhibited any estrogenic activity.

(BASF AG 1998b)

SCCS comment
Despite the lack of clarity on whether uteri were weighed dry or wet and despite the fact that the animals were exposed twice a day, the SCCS agrees that the study results indicate lack of estrogenic activity of benzophenone-4.

3.4.10.4 *In vivo* adverse effects on endocrine relevant endpoints

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SCCS comment
Based on the various studies provided, it can be concluded that benzophenone-4 does not demonstrate endocrine-disrupting properties.

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MoS)

The calculation of the systemic exposure dose (SED) was carried out using data from an *in vitro* percutaneous absorption study, as described in section 3.2.4. As point of departure for risk assessment, a NOAEL of 1239 mg/kg bw/day, based on a 90-day oral repeated dose rat study is used (see section 3.4.4.2). As no good oral absorption study for benzophenone-4 is available, the default value of 50 % is used for the oral absorption, resulting in an adjusted NOAEL of 620 mg/kg bw/day. Following MoS calculations for separate product types and aggregated exposure can be calculated:

Product type	SED (mg/kg bw/day)	adjusted NOAEL (mg/kg bw/day)	MoS
Dermal exposure:			
Sunscreen lotion	0.069	620	8986
Face cream*	0.006	620	103333
Hand cream*	0.008	620	77500
Dermal aggregate for leave-on and rinse-off products	0.067	620	9254
Oral exposure:			
Lip stick*	0.045	620	13778
Inhalatory exposure:			
Sunscreen propellant spray*	0.088	620	7046
Sunscreen pump spray	0.001	620	620000
Overall aggregate* (deterministic)	*0.147*	*620*	*4218*

Considering the different cosmetic products either directly applied on the skin or by spray, at the maximum concentration of 5% benzophenone-4, taken individually and also the aggregated exposure, the MoS is above 100.

3.6 DISCUSSION

Physicochemical properties

The data and information provided on physicochemical properties of benzophenone-4 was incomplete at the first submission. On request, a UV-spectrum was provided and explanations were given with respect to the inconsistent solubility data.
The stability of the test substance in the marketed product (and in the test solutions) was not reported.
A full report of the chemical characterization of benzophenone-4 in terms of purity, identity and impurities in representative batches must be provided and the validity of the analytical methodologies used must be shown. Identity and concentration of any impurities that may be present must also be stated.

Toxicokinetics

A new *in vitro* dermal absorption study report was submitted for benzophenone-4 showing GLP compliance, carried out according to OECD TG 428 and SCCS/1358/10. The range of dermal absorption values varied from 0.05% to 0.56%, with most values leaning towards the lower end of the range. The mean value plus 2 SDs was taken, resulting in 0.46%.

Exposure

Based on the available information, the SCCS considers that the dermal absorption of benzophenone-4 is 0.46% (mean + 2SD) derived from an *in vitro* skin penetration study, except for lip stick where 100% is used. As no good oral absorption study for benzophenone-4 is available, the default value of 50 % is used for the oral absorption. The resulting SED values regarding dermal exposure are: 0.069 mg/kg bw/day (sunscreen lotion), 0.006 mg/kg bw/day (face cream), 0.008 mg/kg bw/day (hand cream), 0.067 mg/kg bw/day (total dermal aggregate for leave-on and rinse-off products); regarding oral exposure: 0.045 mg/kg bw/day (lip stick); regarding inhalatory exposure: 0.088 mg/kg bw/day (sunscreen propellant spray), 0.001 mg/kg bw/day (sunscreen pump spray); and 0.147 mg/kg bw/day for the overall aggregate (deterministic approach).

Toxicological Evaluation

Skin irritation and corrosivity

According to the existing *in vitro* information, neat benzophenone-4 is corrosive for the skin. Diluted in water, benzophenone-4 is non-irritating to rabbits at a concentration of 5% in and up to 10% in humans. This conclusion was reached earlier by the SCCNFP.

Eye irritation and corrosivity

An *in vitro* and *in vivo* eye irritation/ corrosion study were available. Although not carried out according to the Notes of Guidance, they both pointed to eye irritative properties of benzophenone-4, as also indicated earlier by ECHA and to be expected by the acidic pH.

Skin sensitisation

Although animal studies show skin sensitisation potential, for humans the risk of sensitisation to benzophenone-4 can be regarded as very low.

Acute toxicity

The studies cited pointed to low or no acute toxicity of benzophenone-4 in animals. However, it is indicated in the SCCS Notes of Guidance that acute toxicity studies are not mandatory for risk assessment of cosmetic ingredients.

Repeated dose toxicity
From the 90-day repeat dose oral toxicity study in rats, a NOAEL of 1239 mg/kg bw/day could be derived.

Reproductive toxicity
Subject to change whether a typo is present or not in the report of the conducted OECD TG 422 study, no treatment related changes were noted up to 1250 mg/kg bw /day.

Mutagenicity / genotoxicity
Benzophenone-4 has no *in vitro* genotoxic potential.

Carcinogenicity
No data.

Photo-induced toxicity
Benzophenone-4 is not phototoxic, not photosensitising, not photomutagenic and not photoclastogenic.

Human data
Considering available information in humans, the risk of sensitisation is very low.

Special investigation
No indications of endocrine activity.

4. CONCLUSION

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of benzophenone-4, does the SCCS consider benzophenone-4 safe when used as UV-Filter in cosmetic products up to a maximum concentration of 5%?*

On the basis of the safety assessment, and considering the concerns related to potential endocrine disrupting properties, the SCCS is of the opinion that benzophenone-4 is safe when used as UV filter up to a maximum concentration of 5% in sunscreen, face and hand cream, all leave-on and rinse-off products (total dermal aggregate), lipstick, sunscreen propellant spray and pump spray, when used separately or in combination (based on deterministic aggregated exposure).

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of benzophenone-4 in cosmetic products?*

Any additional use of benzophenone-4, such as protectant for stabilising cosmetic formulations when exposed to light, should remain within the concentration mentioned above *(i.e*. 5%), including the use as UV-filter.

3. Does the SCCS have any further scientific concerns with regard to the use of benzophenone-*4 in cosmetic products?*

The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of benzophenone-4 for the environment.

5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158