Scientific Committee on Consumer Safety

SCCS

OPINION on

Benzophenone-3

(CAS No 131-57-7, EC No 205-031-5)

The SCCS adopted this document
at its plenary meeting on 30-31 March 2021

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 2020 until 15 February 2021). Comments received during this time period are considered by the SCCS. For this Opinion, some changes occurred, in particular in sections 3.3.2 biomonitoring data, 3.5 safety evaluation section 'consideration of BP-3 exposure data in humans' and conclusion number 3.

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-3, does the SCCS consider Benzophenone-3 safe when used as a UV-filter in cosmetic products up to a maximum concentration of 6% and up to 0.5% in cosmetic products to protect product formulation?*

   On the basis of safety assessment, and considering the concerns related to potential endocrine disrupting properties of benzophenone-3 (BP-3), the SCCS has concluded that:

   a. The use of BP-3 as a UV-filter up to a maximum concentration of 6% in sunscreen products, either in the form of body cream, sunscreen propellant spray or pump spray, is not safe for the consumer.
   b. The use of BP-3 as a UV-filter up to a maximum concentration of 6% in face cream, hand cream, and lipsticks is safe for the consumer.
   c. The use of BP-3 up to 0.5% in cosmetic products to protect the cosmetic formulation is safe for the consumer.

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Benzophenone-3 as a UV-filter in cosmetic products?*

   In the SCCS's opinion, the use of BP-3 as a UV filter in the following sunscreen products is safe for the consumer up to a maximum concentration of:

   d. 2.2% in body creams, in propellant sprays and in pump sprays, provided that there is no additional use of BP-3 at 0.5% in the same formulation for protecting the cosmetic formulation.
   e. Where BP-3 is also used at 0.5% in the same formulation, the levels of BP-3 used as UV filter should not exceed 1.7% in body creams, in propellant sprays and in pump sprays.

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

   It needs to be noted that the SCCS has regarded the currently available evidence for endocrine disrupting properties of BP-3 as inconclusive, and at best equivocal. This applies to all of the available data derived from *in silico* modelling, *in vitro* tests and *in vivo* studies, either considered individually or taken together. The SCCS considers that, whilst there are indications from some studies to suggest that BP-3 may have endocrine effects, the overall evidence is not conclusive enough at present for the SCCS to ascertain whether or not BP-3 is an ED substance, and this warrants further investigations.

   The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of BP-3 for the environment.

Keywords: SCCS, scientific opinion, Benzophenone-3, UV filter, Regulation 1223/2009, CAS Number 131-57-7, EC No 205-031-5

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background on substances with endocrine disrupting properties

On 7 November 2018, the Commission adopted a review[1] of Regulation (EC) No 1223/2009 on cosmetic products ('Cosmetics Regulation') regarding substances with endocrine disrupting 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 specific 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 then organised a public call for data[2] from 16 May 2019 – 15 October 2019 on 14[3] of the 28 substances (to be treated with higher priority) in order to be able to prepare the safety assessment of these substances. Benzophenone-3 is one of the above-mentioned 14 substances for which the call for data took place.

Existing information on Benzophenone-3

In cosmetic products, the ingredient BP-3 (CAS No 131-57-7, EC No 205-031-5) with the chemical names Oxybenzone, 2-Hydroxy-4-methoxybenzone, (2-Hydroxy-4-methoxyphenyl) phenyl methanone and 2-Benzoyl-5-methoxyphenol is currently regulated as a UV-filter in sunscreen products in a concentration, in ready for use preparation, up to 6 % (Annex VI/4). Furthermore, BP-3 is also allowed in a concentration up to 0.5 % to protect product formulation in all other cosmetic products (Annex VI/4).

Benzophenone-3 has been subject to different safety evaluations by the SCCNFP in 1999[4], and SCCP in 2006 (SCCP/1069/06) and 2008 (SCCP/1201/08). In particular, the last SCCP opinion from 2008 states that '*the use of BP-3 as a UV-filter up to 6% in cosmetic sunscreen products and up to 0.5% in all types of cosmetic products to protect the formulation does not pose a risk to the health of the consumer, apart from its contact allergenic and photoallergenic potential*'.

During the call for data in 2019, stakeholders submitted scientific evidence to demonstrate the safety of BPe-3 as a UV-filter in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on BP-3 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 products_en
[3] Benzophenone-3, kojic acid, 4-methylbenzylidene camphor, propylparaben, triclosan, resorcinol, octocrylene, triclocarban, butylated hydroxytoluene (BHT), benzophenone, homosalate, benzyl salicylate, genistein and daidzein
[4] 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-3, does the SCCS consider Benzophenone-3 safe when used as a UV-filter in cosmetic products up to a maximum concentration of 6% and up to 0.5% in cosmetic products to protect product formulation?*

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Benzophenone-3 as a UV-filter in cosmetic products?*

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

3. OPINION

Safety evaluations of BP-3 have been carried out previously by the SCCNFP (1999) and SCCP (2006 and 2008). This Opinion is based on further safety evaluation that has taken into account the previous SCCNFP/SCCP evaluations, as well as any new relevant information that has since become available, with a particular focus on the potential endocrine disrupting properties of BP-3.

Note: Benzophenone has been quoted in different published studies and articles by different synonyms. These include benzophenone-3 (BP-3); 2-Hydroxy-4-methoxybenzophenone (HMB) and oxybenzone. In the interest of uniformity, the SCCS has referred to it as benzophenone-3 (abbreviated as BP-3) throughout this Opinion.

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

BP-3 is an off-white to light-yellow powder with a melting point of 62°C to 65°C. It is relatively insoluble in water and is readily soluble in most organic solvents. It absorbs ultraviolet (UV) A (320 to 400 nm) and UVB (290 to 320 nm) light, and is photostable. BP-3 produces BP-1 as a biodegradation product under oxygen environment (source: Kim and Choi, 2014; NTP, 2020).

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI name: Benzophenone-3

(SCCP/1069/06)

3.1.1.2 Chemical names

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

(SCCP/1069/06)

IUPAC name
3,3,5-Trimethylcyclohexyl 2 hydroxybenzoate

3.1.1.3 Trade names and abbreviations

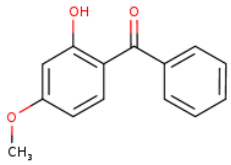
Aduvex 24, Escalol 567, Seesorb 101, Advastab 45, Eusolex® 4360, Spectra-Sorb UV 9, Anuvex, MOB, Sunscreen UV 15, ASL 24, Neo Heliopan BB, Uvasorb MET/C, Chimassorb 90, NSC 7778, Uvinul® M 40, Cyasorb UV 9, Ongrostab HMB, Uvistat 24, Cyasorb UV 9, Light Absorber, Onzone, Viosorb 110.

(SCCP/1069/06)

3.1.1.4 CAS / EC number

CAS No 131-57-7, EC No 205-031-5

3.1.1.5 Structural formula



(ChemIDPlus)

3.1.1.6 Empirical formula

Molecular formula: $C_{14}H_{12}O_3$

(ChemIDPlus)

3.1.2 Physical form

White yellowish, cream coloured powder

(SCCP/1069/06)

3.1.3 Molecular weight

228.26 g/mol

(SCCP/1069/06)

3.1.4 Purity, composition and substance codes

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

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

(SCCP/1069/06)

3.1.5 Impurities / accompanying contaminants

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

(SCCP/1069/06)

3.1.6 Solubility

Water: 0.0037 g/l (20°C), 0.006 g/l (25°C)*

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

Note: These values are taken out of Technical Data Sheets or Material Safety Data Sheets (SCCP/1069/06 and the new safety dossier).

*From ECHA registration dossier (https://echa.europa.eu/el/registration-dossier/-/registered-dossier/5515/4/9/?documentUUID=7f838dc1-dec4-4ae1-b731-b5e6cc1cf30a): EU Method A.6, GLP compliant.

3.1.7 Partition coefficient (Log Pow)

Log Pow: 3.45 at 40°C
> 3.7 (n-octanol/water)
Note: This value is taken from a Material Safety Data Sheet.

(SCCP/1069/06)

3.1.8 Additional physical and chemical specifications

Melting point: 62° - 65°C
Solidification point: 62° - 65°C
Boiling point: > 300°C at 99.86kPa
Loss on drying (40° C): < 2%
Relative density: 1.32 at 25°C, 1.43 at 20°C
Vapour pressure: 0.0011 Pa at 25°C, 0.0005 Pa at 20°C
Particle size distribution (Granulometry): > 100 µm
Ash content at 650°C: 0.1% (upper limit)
Colour number (Gardner): < 4
K-Value: 64 – 67
Odour: almost odourless or faint characteristic
Flash point: > 100°C
Extinction (UV/VIS spectrum in methanol) 400 (0.10 mg/ml cuvette 0.1 cm)
Specific absorbance: 630 - 670 (at 287 nm; 1%, 1 cm, methanol)

Note: These values are taken out of Technical Data Sheets or Material Safety Data Sheet (SCCP/1069/06 and the new applicant dossier).

Additional information from ECHA registration dossier (https://echa.europa.eu/el/registration-dossier/-/registered-dossier/5515/1):

Melting point: 62.9 °C. EU Method A.1, GLP compliant. In practice the melting point is in the range of >62.5 °C to <64.0 °C, typically approx. 63.0 °C.).
Particle size: The inhalable fraction (smaller than 100 µm) is 21%, the thoracic fraction (smaller than 10 µm) is 5.99%, and respirable fraction (smaller than 5.5 µm) is 0.8%. Thus the mass median diameter is larger than 100 µm.

3.1.9 Homogeneity and Stability

Shelf life: at least 2 years
Stability in distilled water: at least 96 hours*
Stability in DMSO: at least 4 hours*
Stability in corn oil: at least 10 days**
Stability in acetone: at least 3 weeks***
Stability in oily lotion: at least 3 weeks***
* determined within recent photomutagenicity studies (full description of stability study under GLP available)
** determined within recent prenatal developmental toxicity study, performed under GLP
*** determined within the oral and dermal US National Toxicology Program (NTP) studies

(SCCP/1069/06)

The 2008 SCCP Opinion (SCCP/1201/08) noted a number of shortcomings in the data provided as part of the dossier assessed in the earlier Opinion (SCCP/1069/06):

'BP-3 is a widespread UV-filter for which over the years a large amount of data have been generated, many of them between 1970 and 1988. This is reflected in the identification and physicochemical data section. The majority of the data are statements from the technical and material data sheets. Also the quality of the toxicological dossier suffers from the fact that studies are often outdated and/or only available as publications in journals, with the result that on several occasions batch number and purity of the test substance are not mentioned, compositions of tested formulations are unknown, etc. Nevertheless the SCCP was of the opinion that the submission summary provided a comprehensive and well-structured overview of the available test descriptions and publications'.

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

3.2.1.1 *In vitro* dermal absorption

The 2008 SCCP Opinion (SCCP/1201/08) concluded that:

'The major shortcoming in the dossier [assessed in SCCP/1069/06] was the absence of a sound dermal absorption study, allowing the calculation of the Margin of Safety for the UV-filter. The newly provided *in vitro* dermal absorption study is considered scientifically acceptable and shows a mean dermal absorption level of 19.3 µg/cm² or 3.1% of the applied dose for a sunscreen (o/w or w/o) containing the maximum requested BP-3 concentration of 6%.'

This study is described below:

Taken from SCCP opinion (2008):

Guideline:	Draft OECD TG 428: Percutaneous Absorption: *in vitro* Method (2000) + SCCP/0970/06
Test system:	Pig ear skin (split-thickness, 400µm) on a flow through diffusion cell
Test substance:	BARNE-40: o/w sunscreen emulsion with BP-3 at 2% BARNE-41: o/w sunscreen emulsion with BP-3 at 6% BARNE-42: w/o sunscreen emulsion with BP-3 at 2% BARNE-43: w/o sunscreen emulsion with BP-3 at 6% (Compositions of sunscreens available in report)
N° of samples:	BARNE-40: 16 samples from 16 donors, (9 evaluable samples) BARNE-41: 14 samples from 14 donors (10 evaluable samples) BARNE-42: 18 samples from 18 donors (10 evaluable samples) BARNE-43: 24 samples from 24 donors (12 evaluable samples)
Batch:	N° 352
Purity:	98% (measured through GC)
Applied amount:	10 µl/cm² (~ 10 mg/cm²), in contact with skin for 24h (no occlusion), rinsed off after 24 hours with either an aqueous solution (BARNE-40-41) or methanol (BARNE-42-43)
Receptor fluid:	20% ethanol in phosphate buffered saline (PBS)
Sampling times:	0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 24 hours
Duration of study:	48 hours
GLP/QAU:	In compliance
Date of test:	Jul - Oct 2007

BP-3 was investigated *in vitro* for its absorption and penetration properties on viable porcine skin by making use of two o/w and two w/o standard sunscreen formulations, containing either 2% or 6% of the UV-filter.

The performing laboratory initiated 3 independent experiments per formulation, each experiment involving measurements on 6 skin samples (18 samples per formulation). Fresh dermatomed pig ear skin samples, with a thickness of 400 ± 80 µm, were treated with 10 µl/cm² of test formulation for 24 hours under dynamic and non-occluded conditions. Blank samples (at 0 hours) were collected immediately after filling the donor chambers at the maximal flow rate of the pump prior to application of the test items. As a measure of skin integrity, the conductivity across the skin samples was determined before treatment and after the last sampling. In addition, positive and negative controls with Benzoic Acid and Basic Red 12, respectively, were used to check the performance of the skin penetration system (historical data, checked every 3-4 months). Washing solutions consisted of either an aqueous solution (for the o/w sunscreens: water + 0.2% acetic acid) or a methanol-based solvent (for the w/o sunscreens: methanol + 0.2% acetic acid). The receptor solution (20% ethanol in PBS) was slowly pumped through the receptor chambers with a flow rate of 0.5 to 2 ml per hour and fractionated 0.5, 1, 2, 4, 6, 8 and 24 hours following the application of each test item. The *stratum corneum* was separated by tape stripping (2 x 5 strips per sample) from the lower skin layers. Analysis for the presence of BP-3 was carried out by HPLC. The use of this HPLC technique for the quantification of BP-3 in various sunscreens was implemented and validated by determining linearity of standard curves, accuracy and precision, sensitivity, specificity, selectivity and stability in the matrix.

Results

Integrity checks of all skin samples lead to the use of skin samples within the acceptable conductivity range of ≤900 µS/cm only. No major impairment of the skin layer was detectable after incubation with the tested sunscreens.

The validation study of the HPLC method revealed a LOD (limit of detection) of 2.0 ng/ml and a LLOQ (lower limit of quantitation) of 2.5 ng/ml in 20% EtOH in PBS and the eluent mixtures. The solubility of BP-3 in the receptor solution was shown to be up to 5 µg/ml.

After the application period of 24 hours, BP-3 was detected in the skin extracts and in the receptor solution samples. The vast majority, however, was found in the wash solution, irrespective of the investigated test item (o/w or w/o sunscreen). The solubilisation of the test items after the application period was noted to be the most critical step regarding an acceptable recovery. Due to the observation that there was an impaired recovery, primarily during the first two experiments, the wash off and extraction procedure was optimized to guarantee a higher recovery in the repetition experiments. As such, 31 samples were excluded from the calculations due to a low recovery (< 85%).

With regard to the measurements in the receptor fluid over time, the amount of BP-3 penetrated during the first 0.5 – 1.0 hours appeared to be negligible (near the LOD of 2.0 ng/ml). Thereafter, there was a constant increase in the portion penetrated through the skin for all formulation types.

Throughout the assay, there was no indication of saturation and no relevant differences were observed with respect to the composition of the test items (o/w versus w/o). The amounts measured at the end of the study in the different compartments are presented in µg/cm² or in % of the applied dose in Table 1 and Table 2, respectively.

As 31 of the 72 samples failed to generate a BP-3 recovery of 85% at the end of the test, the results presented originate from 9 to 12 evaluable samples (each sample coming from a different donor) per formulation tested.

Table 1: Results expressed in µg/cm²

Amount of BP-3	**2% BP-3, o/w (n=9)**	**6% BP-3, o/w (n=10)**	**2% BP-3, w/o (n=10)**	**6% BP-3, w/o (n=12)**
Applied dose	200 ± 10	583 ± 42	201 ± 10	612 ± 37
Receptor fluid	4.5 ± 2.8	6.9 ± 2.1	2.3 ± 0.8	5.9 ± 3.1
Stratum corneum (tape stripping)	1.0 ± 0.7	4.9 ± 10.4	1.3 ± 2.5	8.0 ± 20.0
Epidermis + dermis (24 hours)	3.4 ± 1.6	11.3 ± 11.7	4.4 ± 4.9	13.4 ± 20.5
Washing solution	178 ± 12	512 ± 42	177 ± 14	543 ± 64
Recovery	187 ± 10	535 ± 36	185 ± 10	571 ± 43
Bioavailable portion (RF + epidermis + dermis)	7.9 ± 3.7	18.3 ± 11.3	6.7 ± 4.6	19.3 ± 21.6

Table 2: Results expressed as a % of the applied dose

Amount of BP-3	2% BP-3, o/w (n=9)	6% BP-3, o/w (n=10)	2% BP-3, w/o (n=10)	6% BP-3, w/o (n=12)
Applied dose	100	100	100	100
Receptor fluid	2.3 ± 1.4	1.2 ± 0.4	1.2 ± 0.4	1.0 ± 0.6
Stratum corneum (tape stripping)	0.5 ± 0.4	0.8 ± 1.7	0.6 ± 1.2	1.3 ± 3.2
Epidermis + dermis (24 hours)	1.7 ± 0.8	1.9 ± 1.9	2.2 ± 2.3	2.2 ± 3.2
Washing solution	89.1 ± 4.5	87.8 ± 5.7	88.3 ± 7.6	88.8 ± 9.6
Recovery	93.6 ± 3.9	91.8 ± 4.1	92.3 ± 5.3	93.3 ± 5.0
Bioavailable portion (RF + epidermis + dermis)	4.0 ± 2.0	3.1 ± 1.9	3.3 ± 2.2	3.1 ± 3.4

Conclusion by applicant

The study authors conclude that, under the experimental conditions reported, around 3-4% (between 7-18 µg/cm²) of BP-3 (depending on the concentration in the formulation) penetrated the skin samples during 24 hours and therefore can be considered as bioavailable.

(Chemie Wirtschaftsförderungs GmbH (CWFG, 2007) from SCCP/1201/08)

Other studies:

1) Penetration of 5 sunscreen agents (including BP-3) through human skin was evaluated after application in mineral oil to isolated human epidermal membranes. Following 24h of human epidermal exposure, detectable amounts of all sunscreens were present in the *stratum corneum* and viable epidermis with epidermal penetration most evident with BP-3.

The concentrations of each sunscreen found in human viable epidermis after topical application, adjusting for skin partitioning and binding effects, were at least 5-fold lower, based on levels detected in viable epidermal cells, than those appearing to cause toxicity in cultured human keratinocytes. The IC50 value of BP-3 was 28.8 µM. The authors concluded that the human viable epidermal levels of sunscreens were too low to cause any significant toxicity to the underlying human keratinocytes.

(Hayden et al., 2005 from SCCP/1201/08)

3.2.1.2 *In vivo* dermal absorption studies

1) *In vivo* dermal absorption (human volunteers- Maximal Usage Trial (MUsT))

Guideline:	US FDA. Maximal usage trials for topical active ingredients being considered for inclusion in an over-the-counter monograph: study elements and considerations
Test system:	Human volunteers
Test substance:	BP-3 (formulations containing 4-6%)
Route:	Topical
Dose:	2 mg formulation/cm²; applied to 75% of body surface area
No. of applications:	4 times per day for 4 days
Duration:	7 days
No. of participants:	3/sex/formulation
Metabolite identified:	No
GCP:	Not specified
Study period:	2018

A preliminary clinical study determined whether BP-3 was absorbed into the systemic circulation of 18 healthy participants after topical application of 3 sunscreen products (i.e., 2

aerosol spray products and 1 lotion). 6 participants were randomized per sunscreen. The concentrations of BP-3 were 6% in spray 1, 5% in spray 2 and 4% in the lotion. Two milligrams of sunscreen per cm^2 was applied to 75% of body surface area 4 times per day for 4 days. A total of 30 blood samples were collected at different time points over 7 days. From each participant, plasma concentrations were assessed with validated liquid chromatography with tandem mass spectrometry (LCMS) methods.
Results: All 3 products resulted in detectable plasma concentrations of BP-3 exceeding 20 ng/mL on Day 7. Geometric mean maximum plasma concentrations were 209.6 ng/mL (coefficient of variation (CV): 66.8%) for spray 1; 194.9 ng/mL (CV: 52.4%) for spray 2; and 169.3 ng/mL (CV: 44.5%) for lotion.

Conclusion: application of 3 commercially available sunscreens formulations containing BP-3 at concentrations of between 4-6% under maximal use conditions resulted in systemic absorption and associated plasma concentrations of 169.3 -209.6 ng/mL (Matta et al., 2019).

SCCS comments
Because the percentage of absorption had not been determined, the results from this study have not been used in the safety evaluation.

2) *In vivo* dermal absorption (human volunteers- Maximal Usage Trial (MUsT)

Guideline:	US FDA. Maximal usage trials for topical active ingredients being considered for inclusion in an over-the-counter monograph: study elements and considerations
Test system:	24 human volunteers
Test substance:	BP-3 (4% in lotion-6% in aerosol spray)
Route:	Topical
Dose:	2 mg formulation/cm^2; applied to 75% of body surface area
Application:	0 hours on Day 1 and 4 times on Day 2 through Day4 at 2hour intervals
No. of applications:	13
Duration:	21 days
No. of participants:	12/formulation
Metabolite identified:	No
GCP:	Not specified
Study period:	2019

Topical application of 2 sunscreen products on 12 randomized persons per product. Two milligrams of sunscreen per cm^2 was applied to 75% of body surface area at 0 hour on Day 1 and 4 times on Day 2 through Day 4 at 2 hour intervals. A total of 34 blood samples were collected over 21 days from each participant. *Stratum corneum* samples were collected by tape stripping (6 consecutive strippings) of the lower back (around 3.8 cm^2) on Days 7 and 14. The amounts recovered after tape stripping and the plasma concentrations were assessed with the validated LCMS methods.

Results: Geometric mean maximum plasma concentrations of BP-3 were 258.1 ng/mL (CV: 53%) for lotion and 180.1 ng/mL (CV: 57.3%) for aerosol spray. BP-3 was detectable in skin following tape stripping, with greater amounts detectable on Day 7 compared with Day 14. The continued presence of BP-3 in skin at days 7 and 14, the long terminal half-life typically exceeding 48 hours, and remaining detectable through Day 21 suggest absorption through skin is the rate-limiting step. The levels of BP-3 in skin were 358.5 ng/cm^2 (CV, 194.7%) and 698.5 ng/cm^2 (CV, 155%) on Day 7 and 18.2 ng/cm^2 (CV, 195%) and 29.1 ng/cm^2 (CV, 238.2%) on Day 14 for lotion and aerosol spray, respectively.

Conclusion: the systemic absorption and associated plasma concentrations are between 180.1-258.1 ng/mL. The concentrations in the skin (*stratum corneum*) were in the range of

358.5-698.5 ng/cm² and 18.2-29.1 ng/cm² on Days 7 and 14, respectively. (Matta et al., 2020)

Other studies:

1) The percutaneous absorption and urinary excretion after a whole-body topical application of highly concentrated sunscreen was investigated in 32 volunteers (15 young males and 17 postmenopausal women) treated with 2 mg/cm² of a basic cream formulation on a daily basis for 4 days during the first week, followed by the same treatment regime with a sunscreen containing 30% of UV-filters in total (10% 4-Methylbenzylidene Camphor, 10% BP-3 and 10% Ethylhexyl Methoxycinnamate) during the second week.

Blood concentrations were measured at 0, 1, 2, 3, 4, 24 and 96 h and urine concentrations at 0, 24, 48, 72 and 96 h. All three sunscreens were almost undetectable in plasma and urine before the first application. One to 2 h after the first application, all three sunscreens were detectable in plasma. The maximum median plasma concentrations were 187 ng/ml for BP-3 for females and 238 ng/ml BP-3 for men. In women, urine levels of 44 ng/ml and in men 81 ng/ml BP-3 were found. Thus, all three compounds were detected in their parent forms both in plasma and urine, showing dermal bioavailability.

(Janjua et al., 2008 from SCCP/1201/08)

2) BP-3 applied topically by humans can cross the skin by direct penetration through the intercellular laminae of the *stratum corneum* (SC) or by passive diffusion by high-concentration gradient and then reach the blood (Gonzalez et al., 2006). When 25 volunteers applied a commercially available sunscreen containing 4% BP-3 for 5 days, their urine samples showed that approximately 4% (range 1.2-8.7%, mean 3.7%) of the total amount of BP-3 applied is systemically absorbed (Gonzalez et al., 2006). BP-3 was detected in more than 80% of urine samples of healthy Danish children and adolescents (median concentration 0.92 ng/ml) (Frederiksen et al., 2016). Repeated (4 days) topical applications (2 mg/cm^2 of sunscreen formulation) of BP-3 resulted in urine levels up to 81 ng/ml and plasma levels up to 238 ng/ml (Janjua et al., 2008).

SCCS conclusion on skin penetration
Because the percentage of absorption had not been determined, the results from these *in vivo* studies have not been used in this safety evaluation.

The *in vitro* study presented in the previous SCCP Opinion (SCCP, 2008) is used here as the key study to determine the dermal absorption. The study is carried out according to OECD TG 428 and is part of the data presented by the applicant with respect to dermal absorption the closest to the procedure present in the Notes of Guidance.

In this dermal absorption study, solubilisation problems occurred for the BP-3 application and this resulted in low skin penetration and low total recovery at the end of the experiment. Changes had to be made during the running experiments with respect to the washing and extraction procedures. Of the initial 72 samples at the start of the experiment, only 41 samples showed a total recovery within 85-115% of the initial value. It was argued by the applicant that the failures were in particular noticed in the first two experiments. If for experiment 3 the necessary changes were incorporated in the procedure, it would have been logically consistent to use the results of the third experiment only. This is not the case, as the "good" values of experiments 1 and 2 are also included, but the non-fitting results were discarded.

As the individual data per experiment are not available, the SCCS is of the opinion that it is not acceptable that, out of the 3 experiments, only the fitting data were retained and the remaining data were not considered. Therefore, because of these shortcomings, 2x SD will be added to the mean dermal absorption value measured. Thus, based on the values obtained for 6% BP-3, which is the maximum requested concentration, a dermal absorption of 9.9%

[mean (3.1%) + 2 SD (2*3.4%)] will be used for the calculation of the systemic exposure dose (SED) and the margin of safety (MoS) for sunscreen products. For other cosmetic products, the SCCS will consider a dermal absorption of 8% [mean (4%) + 2 SD (2*2%)] based on the results of the study performed with 2% BP-3.

3.2.2 Other studies on toxicokinetics

Absorption: BP-3 is rapidly absorbed after oral, intravenous, or topical skin administration in rats and piglets (El Dareer et al., 1986; Okereke et al., 1993; Kadry et al., 1995; Kasichayanula et al., 2007; Jeon et al., 2008). In male rats, BP-3, was detected in plasma 5 min after gavage feeding (Okereke et al., 1993) and the absorption half-life was 0.71 h (Kadry et al., 1995).

BP-3 was well absorbed following a single gavage administration of [^{14}C]BP-3 (3.01 to 2,570 mg/kg) in male F344/N rats, with the administered dose excreted primarily *via* urine (63.9% to 72.9%) and feces (19.3% to 41.7%) by 72 hours post-administration. The radioactivity remaining in tissues 72 hours after administration was low (~0.1%) in all dose groups (El Dareer et al., 1986).

Distribution: Once in the systemic circulation, BP-3 is distributed to different organs. Tissue analysis at 6 h after oral administration of BP-3 in male Sprague–Dawley rats showed that BP-1 (major metabolite of BP-3) was present in most tissues including the liver, kidney, testes, intestine, spleen and skin (Okereke et al., 1993). Using the same study design, Kadry et al. (1995) demonstrated that liver contained the highest concentrations of total and free BP-3. In the liver, 6.47% and 0.18% of the initial total and free BP-3 doses were detected, followed by the kidney with 0.97% and 0.02%, respectively. High levels of total and free BP-3 in liver samples imply that the liver is a major organ of BP-3 biotransformation. In hepatocyte suspensions, BP-3 can be converted enzymatically to BP-1 and probably to BP-8 (Nakagawa and Suzuki, 2002).

Metabolism: BP-3 is reported to be metabolised after oral or dermal administration in rats to 2-OH BP and 2,4-diOH BP, with a trace of 2,3,4-triOH BP (Okereke et al., 1993, 1994). Of these metabolites, 2,4-diOH BP (termed as BP-1) has been most frequently detected in rats (El Dareer et al., 1986; Okereke et al., 1993). BP-1 is also the major metabolite in humans (see biomonitoring section). Dermal treatment of Sprague-Dawley rats with BP-3 resulted in detection of its metabolites, including 2,4-diOH BP, in plasma five minutes after administration (Okereke et al., 1994). This metabolite was detected in most tissues within six hours after administration, with the highest concentration observed in the liver (Okereke et al., 1993). Watanabe et al. (2015) detected 5-OH BP-3 as one of the major metabolites in rat and human *in vitro* systems. Maximum plasma concentration of BP-3 was found at 2 h after the topical skin application of BP-3 containing sunscreen products in piglets (Kasichayanula et al., 2007).

Elimination: Conjugation with glucuronic acid and elimination *via* urine is one of the major routes of BP-3 excretion (El Dareer et al., 1986; Kadry et al., 1995; Okereke et al., 1993). Following topical application on skin in piglets, the elimination half-lives of BP-3 ranged between 7.14 and 8.04 h (Fediuk et al., 2012; Kasichayanula et al., 2005, 2007). Prolonged absorption phase through skin may explain longer elimination half-life following topical application.

In a recent study (Mutlu et al., 2019), disposition and metabolism of [^{14}C]BP-3 in rats and mice was assessed following single gavage administration (10, 100, or 500 mg/kg), single IV administration (10 mg/kg), or dermal application (0.1, 1, 10, or 15 mg/kg). Following gavage administration, BP-3-derived radioactivity was well absorbed and excreted mainly in urine (39-57%) and feces (24-42%) with no apparent difference between doses, species or sexes. Distribution of BP-3 in tissues was minimal in rats (0.36%) and mice (<0.55%). Distribution

of BP-3 following dermal application was comparable to that following gavage administration; no differences between doses, sexes, or species were observed but absorption varied between dose vehicles.

SCCS comments
SCCS considers that BP-3 is well absorbed after oral exposure, and therefore will not correct the oral POD used for the MoS calculation to take into account oral bioavailability.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

In cosmetic products, the ingredient BP-3 is currently regulated as a UV-filter in sunscreen products at a concentration up to 6% in ready for use preparations (Annex VI/4). It is also allowed at a concentration up to 0.5% to protect product formulation in all other cosmetic products (Annex VI/4).

Besides cosmetic products and personal care products, BP-3 is also used in the following products: coating products, fillers, putties, plasters, modelling clay and finger paints. Other releases to the environment of this substance are likely to occur from: indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners) and outdoor use as a processing aid (ECHA website).

3.3.2 Biomonitoring data (source: Ruszkiewicz et al., 2017)

Detectable levels of BP-3 have been found in human urine, serum, and breast milk, and it is hypothesised that this is due to sunscreen use (Ruszkiewicz et al., 2017). BP-3 and four other sunscreen filters have been detected in Swiss women's breast milk, indicating that the developing fetus and newborns may also be exposed to these substances (Schlumpf et al., 2008, 2010).

The levels of UV filters found in human samples are usually low. In one epidemiological study, 2517 urine samples from United States (US) general population were analysed for the presence of BP-3, as part of the 2003–2004 National Health and Nutrition Examination Survey (Calafat et al., 2008). BP-3 was detected in 97% of the samples, with mean concentration of 22.9 ng/ml and 95th percentile concentration of 1040 ng/ml.

The European Human Biomonitoring Initiative (HBM4EU) has established a European Union-wide programme to generate knowledge on human internal exposure to chemical pollutants and their potential health impacts in Europe (https://www.hbm4eu.eu/). Under this program, a risk assessment of BP-3 based on internal value was performed recently to compare concentrations of BP-3 and metabolites in urine to health–based HBM-guidance values and to see if part of the EU population is exceeding the reference values. The aim of this work was to exemplify the inclusion of human biomonitoring (HBM) data in risk assessment (RA) strategies. To do so, the available human biomonitoring data on BP-3 was gathered through a systematic review and data from 3 studies. These included BP-3 concentrations measured in urine samples in cohorts from Denmark (Frederiksen et al. (2013), Belgium (Dewalque et al., 2014), and Spain (Adoamnei et al., 2018) that were sampled between 2010 and 2013, before the most recent concentration limit in cosmetic products. The typical cases of BP-3 exposure (based on median values across the studies) ranged from 0.60 to 4.40 µg/g creatinine, with a median of 1.30 µg/g creatinine. The reasonable worst-case BP-3 exposure (based on P95 values across the studies) varied between 16.30 and 392.00 µg/g creatinine, with a median of 33.00 µg/g creatinine.

In another study, investigating correlation between couples' presence of urinary benzophenone-type UV filters and sex ratio of their offspring, the mean concentrations of these compounds ranged from 0.05 ng/ml to 8.65 ng/ml, with BP-3 as the most predominant among the study population (samples collected between 2005 and 2009 in Michigan and Texas)(Bae et al., 2016). Interestingly, about nine times higher than previously reported levels of BP-3 (up to 13000 ng/ ml, average around 200 ng/ml) were found in urine samples collected in 2007–2009 from Californian females, which is probably a result of specific demographics (Philippat et al., 2015).
In placental tissue samples of pregnant women, BP-1 was present in 87.5% (n=16) (Vela-Soria et al., 2011). Frequent detection of BP-3 and BP-1 in breast milk or placental tissues implies potential transfer to the foetus and breastfed infant. Trans-placental transfer ratios of BP-3, i.e., 0.48 (geometric mean), were also suggested by Zhang et al. (2013) employing whole blood samples.

As indicated in the review by Kim and Choi (2014), the urinary levels of BP-3 appeared to be affected by several factors. The urinary concentrations of BP-3 are reported to be higher in females than in males of the U.S.A. (Calafat et al., 2008; Ye et al., 2012) while no gender differences were found in urine of Danish children and adolescents (Frederiksen et al., 2013a). Furthermore, urinary or serum concentrations of BP-3 tend to be higher in urban mothers and in individuals of higher socioeconomic status (SES) (Frederiksen et al., 2013b; Tyrrell et al., 2013), probably reflecting their greater frequency of usage. Differences by sampling countries also have been reported. Detection frequencies of BP-3 appeared to be much higher in the U.S.A. and Europe (80.5–99.0%) than in Asian countries such as Japan (0%, n = 32) and China (25%, n = 100) (Kunisue et al., 2010; Zhang et al., 2013). These results might be attributable to different life styles or to the different pattern of UV filter use among countries. For example, both concentrations and detection rates of BP-3 in personal care products of the U.S.A. (1200 ng/g product weight with 99.1% of detection frequency) were reported higher than those of China (20.1 ng/g product weight, 64.1%) (Liao and Kannan, 2014).

Recent studies indicate that BP-3 exposure may occur from sources other than sunscreens or Personal Care Products (Krause et al., 2012). Frederiksen et al. (2013b) reported BP-3 in almost all urine samples (97–98%) that were collected during seasons when sunscreens are not generally used. In addition, in the river or lakes near WWTP [waste water treatment plant], levels of BP-3 in water were generally higher during spring or fall than in summer (Fent et al., 2010b; Rodil et al., 2008). BP-3 was detected at a similar frequency as adults in the urines of young children aged 6–16 years (Frederiksen et al., 2013a, 2013b). These observations indicate the presence of BP-3 sources other than sunscreen or cosmetics.

Dust ingestion is thought to be one of major sources of BP-3 exposure. Five types of BPs were detected in indoor dust samples from the U.S.A., China, Japan, and Korea, and the detection rates of BP-3 and BP-1 were 100% (Wang et al., 2013). Concentrations of BP-3 in dust were 64.5–1190 ng/g in the U.S.A. and 9.72–1690 ng/g in Korea.

Majhi et al. (2020) have reported median urinary levels of BP-3 in non-pregnant women to be 0.137 µM, whereas in pregnant women it was 0.47 µM, with 95th percentile concentrations in pregnant women being 29.5 µM BP-3.

3.3.3 Calculation of SED

Dermal exposure:

BP-3 as a UV-filter in sunscreens up to 6%- whole body

Description	Parameter	Value	Unit
Applied dose of sunscreen	A	18	g/day
Concentration in the finished product	C	6	%
Dermal absorption (6% formulation)	DA_p	9.9	%
Typical human body weight	bw	60	kg
Systemic exposure dose (SED)	A x 1000 mg/kg x C/100 x DAp/100/bw	**1.78**	**mg/kg bw/day**

BP-3 as a UV-filter in sunscreens up to 6%- face cream

Description	Parameter	Value	Unit
Applied dose of sunscreen	A	1.54	g/day
Concentration in the finished product	C	6	%
Dermal absorption (6% formulation)	DA_p	9.9	%
Typical human body weight	bw	60	kg
Systemic exposure dose (SED)	A x 1000 mg/kg x C/100 x DAp/100/bw	**0.15**	**mg/kg bw/day**

BP-3 as a UV-filter in sunscreens up to 6%- hand cream

Description	Parameter	Value	Unit
Applied dose of sunscreen	A	2.16	g/day
Concentration in the finished product	C	6	%
Dermal absorption (6% formulation)	DA_p	9.9	%
Typical human body weight	bw	60	kg
Systemic exposure dose (SED)	A x 1000 mg/kg x C/100 x DAp/100/bw	**0.21**	**mg/kg bw/day**

BP-3 as a UV-filter at 0.5% to protect cosmetic formulations against sunlight

Description	Parameter	Value	Unit
Applied dose (all cosmetic products)	A	17.79	g/day
Concentration in the finished product	C	0.5	%
Dermal absorption (2% formulation)	DA_p	8	%
Typical human body weight	bw	60	kg
Systemic exposure dose (SED)	A x 1000 mg/kg x C/100 x DAp/100/bw	**0.119**	**mg/kg bw/day**

Inhalation exposure

Using an adapted deterministic 2-box model, the systemic exposure dose through the inhalation route was calculated (Rothe *et al*., 2011).

Description	Parameter	Aerosolised spray	Pump spray	Unit
Amount by application[1]	A	15 000	9000	mg/application
Fraction of BP-3 in non-propellant	C	0.06	0.06	(w/w)
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)	540	108	mg
Near-field, 1 m^2	V_1	1000	1000	L
Breathing rate	BR	13	13	L/min
First step: 2 min in near-field	t_1	2	2	min
Potential amount inhaled during t_1	IA_1 (EA/V_1*BR*t_1)	14.0	2.81	mg
Second step: 10 min in far field	V_2	10000	10000	L
Breathing rate	BR	13	13	l/min
Far-field, 10 m^2	t_2	10	10	min
Potential amount inhaled during t_2	IA_2 (EA/V_2*BR*t_2)	7.02	1.40	mg
Substance availability	G	0.75	0.75	
Respirable fraction	RF	0.2	0.01	
Frequency of application	F	2	2	
Default bodyweight	BW	60	60	kg
SED_{inhal}	$(IA_1*IA_2)*G*RF*F/BW$	**0.1053**	**0.001**	**mg/kg bw/day**

[1]Adjusted for the proportion of propellant (P) to achieve a final "on-body" amount of 9000 mg

It was assumed that for both pump spray and aerosolised spray the same amount of sunscreen needs to reach the skin to ensure the necessary level of sun protection. For an aerosolised 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 for the proportion of non-propellant in formulation based on information by the Applicant, this amount results in an amount of 9 g/application on the skin.

Oral exposure

Description	Parameter	Lip-stick	Unit
Relative daily exposure	$E_{product}$	0.9	mg/kg bw/d
Concentration of BP-3	C	6	%
Retention factor[1]	F_{ret}	100	%
SED_{oral}	$E_{product}*(C/100)*(F_{ret}/100)$	**0.054**	**mg/kg bw/day**

[1]Potential amount available for oral exposure

3.4 TOXICOLOGICAL EVALUATION

3.4.1. Irritation and corrosivity

3.4.1.1 Skin irritation

The SCCP Opinion (SCCP/1069/06) regarded that the 3 reports [indicating benzophenone-3 as non-irritant] were very short (1-2 pages) and provided limited information.

3.4.1.2 Mucous membrane irritation / eye irritation

The SCCP Opinion (SCCP/1069/06) regarded that the 4 reports [indicating BP-3 as non-irritant to rabbit eye] were very short (1-2 pages) and provided only limited information. The Opinion however noted that 'BP-3 is not considered as being irritating to the skin and the eyes. The studies to support this statement are unfortunately outdated and were not performed according to current guidelines and GLP, but the human data with the compound under in-use conditions do not provide any indication of skin and eye irritation due to BP-3. Therefore additional testing in this area does not appear to be necessary.

From new submission (26 August 2020):

In vitro skin irritation (Reconstructed Human Epidermis Test Method)

Guideline:	OECD Test Guideline 439
Test system:	In vitro EPISKIN model / Reconstructed human Epidermitis
Test substance:	Benzophenone-3
Vehicle:	Unchanged
Batch:	Not specified
Purity:	Not specified
Dose applied:	30 µL (25mg)
Concentrations:	Not specified
Negative control:	Dulbecco's phosphate buffered saline (DPBS)
Positive control:	5% sodium dodecyl sulphate (SDS)
Duration of exposure:	60 min
Observation period:	42 hours
GLP:	Yes
Study period:	2019

The skin irritation potential of Benzophenone-3 was investigated in an in vitro assay using EpiDermTM in an OECD Test Guideline 439 compliant study. Undiluted test substance (30 µL) was applied to the EpiDerm™ tissue samples for 60 min followed by an incubation time of 35 min. On the day of exposure, each EpiDerm™ tissue was refreshed with the assay medium, and the test substance including negative control DPBS and positive control 5% SDS were applied to the tissues and analysed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The tissue viability was determined using the EpiDerm™ model based on the enzymatic conversion of the MTT into the purple formazan salt that was quantitatively measured after extraction from viable cells.

Results

The mean tissue viability after treatment with Benzophenone-3 was 97.8%. The tissue viability of the positive control (5% SDS) was 1.6%. The epidermal morphology was comparable to the human epidermis.

Conclusion
Under the conditions of the *in vitro* study, Benzophenone-3 was determined to be non-irritating to skin.

(Han et al., 2019)

3.4.2 Skin sensitisation

The 2008 SCCP Opinion (SCCP/1201/08) noted both a guinea pig Magnusson Kligman Maximisation test (GPMT) and an LLNA indicated that BP-3 is non-sensitising in these experimental models. In addition, the 2005-submission contained a number of reports of clinical trials with regard to the photoallergenic potential of UV-filters in general. In each of those, a number of clear positive reactions to BP-3 were described. The SCCP added a number of references and emphasised that, looking at the positive photoallergic reactions to BP-3, one must keep in mind that the study population in all tests consisted of patients with a suggested history of photocontact allergy. Taking all the information together, the SCCP concluded that BP-3 can cause photoallergic reactions.

SCCS comments
Taken together the clinical data considered in the 2008 SCCP Opinion, the results from more recent studies (e.g. Beleznay et al., 2014; Hanson & Warshaw, 2015; Haylett et al., 2014; Spiewak, 2013; Subiabre-Ferrer et al., 2019; Valbuena Mesa & Hoyos Jiménez, 2016; Warshaw et al., 2013) and the outcome of pre-marketing human repeated insult testing of products containing BP-3 (NTP, 2020), confirm the previous evaluation of the SCCP that BP-3 can cause photoallergic reactions. The European photo-patch test task force has stated that BP-3 is the second most frequent photo contact allergen among the UV filters (Gonçalo, 2013). In addition, one case of anaphylactic reaction to BP-3 has been reported (Tawfik & Atwater, 2019).

3.4.3 Acute toxicity

The 2006 SCCP Opinion (SCCP/1069/06) stated that the studies reporting the acute toxicity values in terms of LD50-oral-rat (>6,000, 11,600 and >12,800 mg/kg), and LD50-dermal-rabbit (>16,000 mg/kg) were not performed according to appropriate guidelines and GLP practices.

3.4.3.1 Acute oral toxicity

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3.4.3.2 Acute dermal toxicity

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3.4.3.3 Acute inhalation toxicity

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3.4.4 Repeated dose toxicity

The 2008 SCCP Opinion (SCCP/1201/08) noted that the studies provided as part of the dossier assessed in the earlier Opinion (SCCP/1069/06) had indicated that:

'After repeated oral administration of BP-3 in rats and mice, the most frequently encountered adverse effects consisted of unspecific signs of systemic toxicity in the form of reduced food consumption and retarded body weight gain, together with effects on the identified target organs being the kidney and the liver. These effects were partly associated with changes in clinical chemistry. Very often the most susceptible parameter was the increase in liver weight. The latter, however, without any histopathological correlate, is not considered by the submission authors to reflect an adverse effect per se but should be considered as an adaptive metabolic response which is known to be reversible. Therefore, according to the 2005-submission, the oral NOAEL corresponded to 411 mg/kg bw/day. With regard to the results of the dermal repeated dose studies, a dermal NOAEL of 200 mg/kg bw/day was put forward, on the assumption that deviations without dose-response relationship and without correlated histopathological findings (e.g. the decreased reticulocyte count, increased relative kidney weight, increased platelet count and whole blood cell count in the 90d dermal study in rat) should not be taken into account.'

The studies already included in the previous SCCS Opinion are not detailed in this Opinion; if they provide information on ED effects, they will be described in the section 3.4.9.

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

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

The National Toxicology Program (NTP) has assessed the potential adverse effects of sunscreens in human-relevant model systems. The scope of studies relating to BP-3 includes the assessment of potential endocrine activity under the U.S. Environmental Protection Agency Endocrine Disruptor Screening Program Phase 1 studies, and characterisation of the potential effects of continuous exposure over multiple generations using the NTP Modified One-Generation study design.

Fourteen-week Interim Evaluation

This study is a part of the 2-year toxicity study described below (section 3.4.5 and 3.4.7)

Groups of 10 male and 10 female rats were exposed to 0 or 10,000 ppm BP-3 for 14 weeks.

At the 14-week interim evaluation, the mean body weight of the 10,000 ppm males was not significantly different than that of the control males, but the mean body weight of the 10,000 ppm females was significantly decreased and was approximately 87% that of the control group at 14 weeks. In males, the absolute and relative liver and right kidney weights were increased in the 10,000 ppm group compared to the control group. In females, the absolute kidney weight was significantly decreased, and the relative liver weight was significantly increased relative to the control group. Other changes in organ weights were not considered toxicologically relevant.

The incidence of mixed-cell cellular infiltration in the liver was significantly increased in 10,000 ppm males relative to the control group. These cellular infiltrates were composed of mononuclear cells with scarce neutrophils and had no specific predisposition to a specific area

of the liver lobule. It is unlikely that the cellular infiltrates, which were all of minimal severity, would be responsible for the changes in the liver weights observed in male rats at this time point. No other histologic findings were observed to explain the differences in organ weights, but in the females, body weight changes could have influenced the absolute kidney weight decrease and the relative liver weight increase. However, the increase in relative liver weight in exposed females was accompanied by an insignificant absolute liver weight increase, so it is unlikely that body weight was responsible for the liver weight changes.

As a part of the 14-week interim evaluation in the F1 rat study, transcriptome analysis was performed on RNA extracted from microarray study male rat livers from the 10,000 ppm and control groups. The observed effects on transcription were consistent with a mild induction of xenobiotic metabolism-related processes that is likely related to the observed relative liver weight increase. Analysis of a subset of estrogen-responsive genes showed no change in response to BP-3.

3.4.4.3 Chronic (> 12 months) toxicity

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3.4.5 Reproductive toxicity

The 2008 SCCP Opinion (SCCP/1201/08) noted that 'the available teratogenicity study in the rat generated a NOAEL-value for maternal and developmental toxicity of 200 mg/kg bw/day, which was used to calculate the MoS '. The NOAEL value is derived from a 2005 prenatal developmental toxicity study (https://echa.europa.eu/el/registration-dossier/-/registered-dossier/5515/7/9/3).

Other reprotoxicity studies have been published since the release of the previous SCCS opinion. The more informative studies are described below and summarised in Annex-1.

The study from Nakamura et al. (2015) brings information of the reprotoxicity of BP-3 and also on some ED properties. This study is therefore described in this section and also in more detail in the ED section 3.4.9.

Nakamura et al. (2015) determined the effects of maternal and lactational exposure to BP-3 on development and reproductive organs of offspring of time-mated female Harlan Sprague-Dawley rats (11- to 13-week-old; seven to eight per group). Pregnant dams were housed individually and maintained under a 12 hr/12 hr light/dark cycle with controlled room temperature (23 ± 3°C) and humidity (50 ± 20%). Starting from gestation day 6 until weaning on postnatal day (PND) 23, dams were fed low-phytoestrogen chow containing 0, 1000, 3000, 10,000, 25,000, or 50,000 ppm BP-3. From the amounts of feed consumed, the authors calculated average consumed doses as 0, 67.9, 207.1, 670.8, 1798.3, and 3448.2 mg/kg/day respectively.
The results showed reduced organ (liver, kidney) weights in female and male offspring and blood biochemical factors in the high dose (≥10,000 ppm) groups. There was no significant difference in the number of implantation sites/litter, mean resorptions/litter, % litters with resorptions, number and weights of live fetuses, or sex ratios between the control and treated groups. In general, exposure to <10,000 ppm BP-3 was not associated with adverse effects on the reproductive system in rats.

Some effects were observed in the highest dose groups. These included a decrease in the normalised anogenital distance in male pups at PND 23; impairment of spermatocyte development in testes of male offspring; delayed follicular development in females. The authors concluded that the doses at which the adverse effects were observed were much higher than usual human exposure levels.

Recent data from NTP (2020)

Guideline:	ICH S5(R2) 4.1.3 (ICH, 1993); similar or OECD test guideline 414
Species/strain:	Rat / Sprague-Dawley
Group size:	25 pregnant female rats/group
Test substance:	BP-3
Batch:	1F100604
Purity:	99% (HPLC-PDA analysis)
Dose levels:	0, 3000, 10000 or 30000 ppm equivalent to 242, 725 and 3689 mg/kg bw/day
Route:	Oral
Administration:	Dietary
Exposure period:	From gestation Day 5 to gestation Day 15
GLP:	Yes
Study period:	2012 (in-life portion completed in 2012; final report issued in 2015)

Dams did not display any adverse clinical findings before or after parturition. Gestation body weights of the dams were statistically significantly decreased (approximately 3%) at 10000 ppm. Dams at 10000 ppm showed slight but statistically significant reduced mean body weights on GD 9 (2.7%), GD 18 (2.5%), GD 21 (3.3%) and on lactation days 1 (4.2%), 7 (5.3%), 14 (3.4%) and 21 (2.7%) compared to the control group. The body weight gains were significantly decreased during GD6-9 (46%) and lactation day 4-21 (43%). Dams at 3000 ppm showed similar slight but significant decreased mean body weight at GD 21 (3.4%), lactation days 1 (2.6%), 7 (3.4%) and 21 (3.0%) compared to the control group. However, the body weight gains were reduced only during GD 18–21 (14%), and similar effects were not seen during the lactation period. Lower body weight gain over the GD 6–9 (10000 ppm) and 18–21 (3000 and 10000 ppm) intervals, which was associated with slightly lower feed consumption over the GD 18–21 interval, likely contributed to this response. There were no effects on the percentage of mated females producing pups, litter size, pup sex distribution, or on numbers of male or female pups. The apparent decrease in the percentage of females pregnant in the 10000 ppm group was attributed to the 7 animals that had no evidence of pregnancy, evidenced by an absence of implantation sites. Therefore, the lower pregnancy rate was not considered to be exposure related given that exposure began after implantation. Litter size of the 10000 ppm dose group was slightly lower on PND 7 and 10 (7.82 compared with 8.00 in control and other treated groups). However, at PND 1, 14, 17 and 21 there were no statistical differences. At PND 21 live litter size at 10000 ppm was 7.82 compared with 7.91, 7.97 and 7.93 in the control, 1000 ppm and 3000 ppm groups respectively. This difference in litter size on PND 7 and 10 is therefore considered to be unrelated to treatment. Male, female, and combined pup body weights were not statistically significantly different across the exposure groups on PND 1. However, on PND 4, pup body weights (male, female, and both) in the 10000 ppm exposure groups were statistically significantly decreased (both sexes approximately 5% lower) compared to the control group. This response was generally observed over subsequent pre-weaning weights, and by PND 21 combined live pup weight at this dose was 8% lower than control.

Conclusion from the applicant

Under the conditions of this study, the maternal NOAEL is 3000 ppm (206-478 mg/kg bw/day), based on reduced body weight gain during GD 6–9 and lactation day 4–21. The developmental NOEL is considered as 3000 ppm (206-478 mg/kg bw/day) based on impaired postnatal bodyweight performance at 10000 ppm (660-1609 mg/kg bw/day).

(National Toxicology Program, 2020)

Guidelines/Guidances:	NTP Modified One-Generation study design
GLP:	FDA GLP

Test item:	2-Hydroxy-4-methoxybenzophenone = BP-3
Purity:	> 99%
Vehicle:	In feed
Route of exposure:	Oral
Duration of exposure:	F0 female from GD 6 until PND21
Doses:	0, 1000, 3000 and 10000 ppm equivalent to of approximately 70, 206, and 660 mg BP-3/kg body weight/day during gestation, and 157, 478, and 1,609 mg/kg/day over lactation days (LD) 1–14
Experimental animals:	
Species:	Rats
Strain:	Sprague Dawley (Hsd:Sprague Dawley® SD®)
Sex:	Female
Animal numbers:	Between 35 and 42 females for F0

In this study design, exposure to BP-3 in the diet began on gestation day (GD) 6. At weaning, one and two pups per sex per litter were allocated to prenatal and reproductive performance cohorts, respectively. In addition to an assessment of reproductive performance, F2 fetal outcomes (GD 21 fetal examinations) were assessed in one cohort and the potential effects on parturition and early growth of the F2 generation were assessed in the other cohort. Internal dose metrics were also assessed. Apical indicators sensitive to endocrine modulation were measured (e.g., anogenital distance, thoracic nipple retention, pubertal indices, reproductive tissue histology). The U.S. Food and Drug Administration's National Center for Toxicological Research (NCTR), in partnership under an Interagency Agreement, has also examined the effects of maternal and lactational exposure to BP-3 on development and reproductive organs in male and female rat offspring, and on transcriptional changes in the testes and prostates of young rats.

Results

Gestation body weights of dams receiving 10,000 ppm BP-3 in the diet were slightly lower (~3%) than those of the control group and showed statistically significant differences. Dams receiving 3,000 or 10,000 ppm BP-3 in the diet displayed slight decreases in GD 6–21 body weight gain (~10%) relative to the control group that attained statistical significance. Lower body weight gain over the GD 6–9 (10,000 ppm) and 18–21 (3,000 and 10,000 ppm) intervals, which was associated with slightly lower feed consumption over the GD 18–21 interval, likely contributed to this response. These collective effects are minimal and would not be sufficient to affect the normal development of the offspring.

Administration of BP-3 had no effect on the percentage of mated females producing pups, litter size, pup sex distribution, or numbers of male or female pups. The apparent decrease in the percentage of females pregnant in the 10,000 ppm group can be attributed to the seven animals that had no evidence of pregnancy (i.e., not exposure-related given that exposure began after implantation). Dams receiving BP-3 did not display any adverse clinical findings before or after parturition. Litter size of the 10,000 ppm BP-3 group was slightly lower on PNDs 7 and 10.

Male, female, and combined pup body weights were not significantly different across the exposure groups on PND 1. However, on PND 4, pup body weights (male, female, and both) in the 10,000 ppm exposure groups were ~10% lower than those of the control groups, and this response was generally observed over subsequent pre-weaning weights.
Pups were weaned on PND 21, which was considered day 1 of the 2-year exposure period.

(National Toxicology Program, 2020)

3.4.6 Mutagenicity / genotoxicity

The 2008 SCCP Opinion (SCCP/1201/08) noted that the studies provided as part of the dossier assessed in the earlier Opinion (SCCP/1069/06) had indicated that:

'As far as the (photo)mutagenic/(photo)genotoxic potential of BP-3 was concerned, the presented *in vitro* and *in vivo* assays indicated that the substance did not possess (photo)mutagenic of (photo) genotoxic properties.'

The National Toxicology Program (NTP) of the U.S. Department of Health and Human Services has published a draft technical report (see below section 3.4.7). Results of bacterial mutagenicity tests conducted using standard testing approaches with the same lot of BP-3 tested in the 2-year studies were negative in TA98 and TA100, as well as in Escherichia coli strain WP2 uvrA pKM101, with and without rat liver S9.

New study on Mammalian Cell Gene Mutation Test in Chinese hamster lung fibroblasts (HPRT locus):

Guideline:	OECD Test Guideline 476
Test system:	Chinese hamster lung fibroblasts (V79), HPRT locus
Replicates:	Duplicates
Batch:	Not specified
Purity:	Not specified
Concentrations:	8.92 – 2283 µg/mL
Preliminary test (range finder):	±S9 mix: 8.92, 17.84, 35.67, 71.34, 142.69, 285.38, 570.75, 1141.5 and 2283 µg/mL
Main test:	
Experiment 1:	5, 10, 20, 40, 80, 120 and 160 µg/mL without S9-mix 5, 10, 20, 40, 60, 80 and 160 µg/mL with S9-mix
Experiment 2:	5, 10, 20, 40, 60, 80, 120 and 160 µg/mL for both exposure groups
Exposure duration:	4 and 24 hours
Expression time:	7 days
Vehicle:	DMSO
Positive controls:	
With S9-mix:	7,12-dimethylbenzanthracene
Without S9-mix:	ethylmethanesulphonate
GLP:	Yes
Study period:	2013

BP-3 was tested in an OECD Test Guideline 476 compliant study to investigate mutagenic potential at the HPRT locus (6-thioguanine resistance) in V79 Chinese hamster lung fibroblasts. The study consisted of a cytotoxicity range finder followed by two independent experiments, each conducted in the presence and absence of S9-mix.
A preliminary cytotoxicity experiment was performed on cell cultures with the dose levels ranging from 8.92 to 2283 µg/mL (separated by 2-fold intervals) in the presence and absence of S9-mix. Results from the preliminary cytotoxicity test were used to select the test substance dose levels for the mutagenicity experiments.

The main study was performed in two independent experiments, using two parallel cultures each. Test substance treatments were performed for 4 hours exposure period both with and without S9-mix at 5 dose levels (5-160 µg/mL), vehicle and positive controls. All doses were plated to determine viability and 6-thioguanine resistance 7 days after treatment. In the repeat experiment, the same dose range with eight dose levels (5, 10, 20, 40, 60, 80, 120, and 160 µg/mL) was used with 4 hour and 24 hour exposure in the presence and absence of S9-mix, respectively, and with vehicle and positive controls.

Results

Precipitation and cloudy precipitate of the test substance were seen at the end of the exposure period in the absence of S9-mix at 160 µg/mL and cloudy precipitate was seen at the end of the exposure period as of 40 µg/mL. Precipitation was seen at 160 µg/mL in the presence of S9-mix.

Mutant frequencies (MF) in vehicle control cultures fell within acceptable ranges and clear increases in mutation were induced by the positive control treatment with and without S9-mix. Therefore, the study was accepted as valid.

No statistically significant increases in mutant frequency were observed following treatment with test substance at any concentration tested in the presence or absence of S9-mix in both independent experiments.

Conclusion

Under the conditions of the study, BP-3 did not induce mutations at the HPRT locus of V79 cells in the presence or absence of S9-mix.

(ECHA (European Chemical Agency) REACH dossier (2020). Registered substances database: Oxybenzone, EC No. 205-031-5. CAS No. 131-57-7, Last modified: 27 Feb 2020)

In a recently published study (Majhi et al., 2020), in human breast epithelial cells, DNA damage following treatment with BP-3 was determined by immunostaining with antibodies against gamma-H2AX and 53BP1. Formation of R-loops was determined with DNA:RNA hybrid–specific S9.6 antibody. Short-term exposure to the chemicals was also studied in ovariectomized mice. Immunostaining of mouse mammary epithelium was performed to quantify R-loops and DNA damage in vivo. As a result, concentrations of 1 µM or 5 µM BP-3 increased DNA damage similar to that of E2 treatment in a ERα-dependent manner. BP-3 exposure caused R-loop formation in a normal epithelial cell line when ERα was introduced. R-loops and DNA damage were also detected in mammary epithelial cells of mice treated with BP-3.

SCCS comment
The biological meaning of the results of study is not clear at the moment. Although proof of the formation of R-loops and DNA double strand breaks was provided after exposure to BP-3, it is not certain how this translates into a mutagenic hazard of BP-3.

Overall SCCS conclusion
As noted in the SCCP/1201/08 Opinion, the newly provided data on gene mutations at the HPRT locus in V79 cells also support the safety of BP-3 in terms of genotoxicity.

3.4.7 Carcinogenicity

The National Toxicology Program (NTP) of the U.S. Department of Health and Human Services has published a draft technical report finding that ultraviolet ray-absorbing ingredient 2-hydroxy-4-methoxybenzophenone (HMB; BP-3; CAS 131-57-7) provides "equivocal evidence of carcinogenic activity" in rats. This level of evidence is the third level within five categories and is defined as representing "a marginal increase of neoplasms that may be chemical related."

BP-3 was nominated to NTP by the National Cancer Institute because of high exposure via use of BP-3-containing sunscreen products and lack of carcinogenicity data.

Two-year studies were completed in both mice and rats, exposing them to concentrations between 0 and 50,000 ppm via food. It was found that exposure to BP-3 in rats led to an increase of non-neoplastic lesions of the testis and pancreas in males and of the uterus and adrenal cortex in females. Increases in lesions were also observed in the bone marrow, spleen, and kidney of both sexes in mice.

Rat study

Guidelines/Guidances:	NTP Modified One-Generation study design
GLP:	FDA GLP
Test item:	2-Hydroxy-4-methoxybenzophenone = BP-3
Purity:	> 99%
Vehicle:	In feed
Route of exposure:	Oral
Duration of exposure:	2 –years beginning on gestation day (GD) 6
Doses:	0, 1000, 3000 and 10000 ppm equivalent to 58, 168, and 585 mg BP-3/kg body weight for males and 60, 180, and 632 mg/kg for females
Experimental animals:	
Species:	Rats
Strain:	Sprague Dawley (Hsd:Sprague Dawley® SD®)
Sex:	Male and female
Animal numbers:	Between 35 and 42 female for F0 and 50 and 60 per sex for F1
Study period:	2010-2012

Beginning on gestation day (GD) 6, groups of 42, 35, 35, and 43 F0 time-mated female rats were fed diets containing 0, 1,000, 3,000, and 10,000 ppm BP-3, respectively, for 39 days. Groups of 50 (1,000 and 3,000 ppm) or 60 (0 and 10,000 ppm) F1 rats per sex continued on study after weaning and were fed diets containing the same exposure concentrations for 105 weeks; 10 F1 rats per sex from the 0 and 10,000 ppm groups were evaluated at 14 weeks. Dietary concentrations of 1,000, 3,000, and 10,000 ppm resulted in average daily doses of approximately 58, 168, and 585 mg BP-3/kg body weight for males and 60, 180, and 632 mg/kg for females.

Results:

Survival of all exposed groups of F1 male and female rats was not significantly different than that of the control groups. Over the course of the study, mean body weights of F1 males and females in the 10,000 ppm exposure groups were 10–25% lower than those of the control groups. After week 77, F1 female mean body weights in the 3,000 ppm exposure group were 10% lower than those of the control group. Feed consumption by exposed groups of F1 males and females was generally similar to that by the control group throughout the study.

In the brain and spinal cord, the occurrence of malignant meningiomas in males at the end of the 2-year study was 0/50, 1/50, 4/50, and 0/50.

In the thyroid gland, the incidence of C-cell adenoma in 3,000 ppm females was significantly greater than that in the control group at the end of the 2-year study.

In the uterus, the incidence of stromal polyp in 3,000 ppm females was significantly increased. A significantly increased incidence of atypical endometrium hyperplasia of the uterus also occurred at 3,000 ppm; however, the incidence of adenocarcinoma was significantly decreased in this group.

In the adrenal cortex, the incidences of focal hypertrophy were significantly increased in 1,000 and 3,000 ppm females at the end of the 2-year study.

In the testes, the incidence of fibrinoid necrosis of the arterioles was significantly increased in 10,000 ppm males at the end of the 2-year study, and there was an increasing trend in the incidence of interstitial cell hyperplasia.

(National Toxicology Program, 2020)

Mice Study

Guidelines/Guidances:	NTP guidelines
Test item:	2-Hydroxy-4-methoxybenzophenone = BP-3
Purity:	> 99%
Vehicle:	In feed
Route of exposure:	Oral
Duration of exposure:	2 –years
Doses:	0, 1000, 3000 and 10000 ppm equivalent to 113, 339, and 1,207 mg BP-3/kg body weight for males and 109, 320, and 1,278 mg/kg for females)
Experimental animals	
Species:	Mice
Strain:	B6C3F1/N mice
Sex:	Male and female
Animal numbers:	50 males and 50 females per dose group
GLP:	FDA GLP

Groups of 50 male and 50 female mice were fed diets containing 0, 1,000, 3,000, or 10,000 ppm BP-3 (equivalent to average daily doses of approximately 113, 339, and 1,207 mg BP-3/kg body weight for males and 109, 320, and 1,278 mg/kg for females) for 104 (females) or 105 (males) weeks.

Results:

Survival of all exposed groups of male and female mice was not significantly different from that of the control groups. Mean body weights of 1,000 and 3,000 ppm males and females were within 10% of those of the control groups throughout the study. Mean body weights of 10,000 ppm males and females were at least 10% lower than those of the control groups generally after weeks 69 and 17, respectively. Feed consumption by exposed groups of males and females was not significantly different from that by the control groups.

The incidences of pigment in the bone marrow were significantly increased in 10,000 ppm males and females. The incidences of pigment in the spleen were significantly increased in 10,000 ppm males and 3,000 and 10,000 ppm females.

In the liver, the incidence of hepatocyte syncytial alteration was significantly increased in all exposed groups of males.

In the kidney, the incidences of chronic progressive nephropathy were significantly increased in 3,000 and 10,000 ppm males. In addition, the incidences of renal tubule cytoplasmic alteration and lymphocytic cellular infiltration were significantly increased in 10,000 ppm males. The incidence of osseous metaplasia was significantly increased in 10,000 ppm females compared to the control group.

Conclusion from NTP:

Under the conditions of these 2-year studies, there was equivocal evidence of carcinogenic activity of BP-3 exposure in male Hsd:Sprague Dawley® SD® rats based on the occurrence of brain and spinal cord malignant meningiomas. There was equivocal evidence of carcinogenic activity in female Hsd:Sprague Dawley® SD® rats based on the increased incidence of thyroid C-cell adenomas and the increased incidence of uterine stromal polyps. There was no evidence of carcinogenic activity in male or female B6C3F1/N mice at exposure concentrations of 1,000, 3,000, and 10,000 ppm.

Increases in the incidences of non-neoplastic lesions of the testis and pancreas in male rats and of the uterus and adrenal cortex in female rats occurred with exposure to BP-3. Increases in the incidences of non-neoplastic lesions of the bone marrow (males and females), spleen (males and females), kidney (males and females), and liver (males) in mice occurred with exposure to BP-3.

(National Toxicology Program, 2020)

SCCS comments
The SCCS concurs with the NTP and considers that BP-3 has 'equivocal evidence of carcinogenic activity'.

3.4.8 Photo-induced toxicity

3.4.8.1 Phototoxicity/photoirritation and photosensitisation

Taken from SCCP 2006

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

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

In the case of BP-3, the presented publications clearly indicate that the UV-filter is a photoallergen."

In vitro 3T3 (NRU) phototoxicity test

Guideline:	Not specified (precursor of OECD-432)
Test system:	Mice Balb/c 3T3 cells
Test substance:	BP-3
Batch:	Not specified
Purity:	Not specified
Vehicle:	Aqueous Dimethyl sulfoxide
Positive control:	Chlorpromazine
Exposure duration:	24 hours
Concentrations:	100-10000 µg/mL

Irradiation:	5 J/cm² (UVA) and 30 mJ/cm² (UVB)
GLP:	Not specified
Study period:	1998

BP-3 was included in the battery of UV filters employed in the ECVAM validation 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT) to check the accuracy and repeatability of the proposed in vitro assay aiming to evaluate the photo-toxicity of tested substances. Balb/c 3T3 mice fibroblasts were incubated for 60 minutes with several concentrations (from 100 to 10000 µg/mL) of the test compound. Thereafter, the cells were exposed to a sun simulator for 50 minutes. After 24 hours, the neutral red uptake (NRU) was measured and the respective EC50 values were defined as the concentrations of the test substance that cause 50% reduction of NRU compared to the untreated control cultures. Subsequently the Photo Irritation Factor (PIF) was calculated by comparing two equally effective cytotoxic concentrations (EC50) of the test chemical obtained in the absence (-UV) and in the presence (+UV) of a noncytotoxic irradiation. Substance showing PIF ≥ 5 are considered phototoxic. Additionally, Mean Photo Effect (MPE) was calculated by a special computer software based on comparison of the complete concentration response curves. Substances showing MPE ≥ 0.1 were considered phototoxic.

Results
BP-3 was shown to be below the respective cut-off criteria for phototoxicity with the exception of one single PIF value of >2.7 and one MPE value of 0.195 obtained by a single laboratory (upon 11). This single event was considered as incidental.

Conclusion
BP-3 was not phototoxic in the 3T3 NRU assay in the presence or absence of artificial sunlight.

Spielmann et al., 1998

SCCS comments
The test was performed in the framework of a validation study for OECD-432.
Benzophenones (including BP-3) were evaluated by 8 laboratories. The results indicate that BP-3 is not phototoxic.

3.4.8.2 Photomutagenicity / photoclastogenicity

Taken from SCCP 2006

"As far as the (photo)mutagenic/(photo)genotoxic potential of Benzophenone-3 is concerned, the presented *in vitro* and *in vivo* assays indicate that the substance does not possess (photo)mutagenic of (photo) genotoxic properties."

No more recent data have been made available to SCCS, therefore SCCS considers that this conclusion is still valid.

3.4.9 Special investigations: assessment of endocrine disrupting potential

- BP-3 has not been identified under REACH regulation as a SVHC for its ED properties either for the environment or for human health (ECHA website https://echa.europa.eu/candidate-list-table consulted on 02 December 2020).
- Danish Centre: Evaluation: Suspected ED in Category 2a (Hass et al., 2012).

- In February 2019, FDA considered BP-3 in category III: not safe for use as UV filter. FDA considered that inadequate data on BP-3's developmental and reproductive toxicity were reported. The literature suggests that BP-3 may have endocrine activity (see, e.g., Schlumpf et al., 2001; Schlumpf et al., 2004; Krause et al., 2012).

3.4.9.1 Endocrine Mode of Action

- (anti)-Estrogenic Disrupting Effects

In vivo

In rodents, BP-3 was reported to be slightly active in the uterotrophic assay using immature Long-Evans rats (ED50: 1,000 to 1,500 mg/kg per day) (Schlumpf *et al.*, 2001). However, BP-3 did not cause a uterotrophic response in ovariectomized rats when tested up to 1 g/kg in an NTP study (Appendix F, NTP report, 2020).

In vitro

BP-3 was shown to have some estrogenic activity on a recombinant yeast carrying the estrogen receptor of rainbow trout (rtERalpha) but BP-3 did not show any induction potential of vitellogenin after 14 days of aqueous exposure *in vivo* in fathead minnows (Kunz *et al*., 2006).

According to the review by Kim and Choi (2014), the binding affinity of BP-3 to ER appears to also vary depending on the ER subtype, with higher affinity for ERα comparing to ERβ (Molina-Molina *et al*., 2008; Schreurs *et al*., 2002, 2005). All BP derivatives tested except BP-3 were full hERα and hERβ agonists (BP2>THB>BP1) and displayed a stronger activation of hERbeta compared with hERalpha, the opposite effect to that of estradiol (E2). All four BP derivatives showed also anti-androgenic activity (THB>BP2>BP1>BP-3) (Molina-Molina *et al*., 2008).

In the studies from Schreurs *et al.* (2002, 2005), BP-3 was found to be a full ER alpha agonist in transfected HEK293 cells with hERa and hERb genes. BP-3 was also able to activate transcription through ERb to significant levels. In HEK293 cells, the EC50 value of transactivation for hERα was one order of magnitude lower than for hERβ (Schreurs *et al.,* 2005). ERα activation is related to the cell proliferation while ERβ is reported to play an important role in cell differentiation.

In another *in vitro* study, **Blair et al. (2000)** determined BP-3 to be an ER non-binder in a rat uterine cytosolic ER competitive binding assay (IC_{50} >1.00 x 10^{-4} M). The *in vitro* assay was carried out using [^{3}H]-E_2 in a uterine cytosol preparation from excised uteri from adult non-pregnant Sprague-Dawley rats. All assays were replicated at least twice. The authors considered that, from a structural point of view, the results could be explained by the presence of a 4-hydroxyl and phenolic groups, and that chemicals with suitable molecular weights can be expected to fit the binding pocket of ER and an electronegative atom or group increases binding interaction with ER.

Schlecht et al. (2004) studied the effects of BP-3 on the expression pattern of the estrogen receptors ERα and ERβ, the estrogen receptor-related receptor-1 (ERR1), and the aryl hydrocarbon receptor (AhR) in adult ovariectomized rats. The study involved 5-day treatment of rats at two dosages of BP-3, with the use of a 17-estradiol (E2) and a negative control group. The expression of four receptor mRNAs (ERα, ERβ, the structurally related estrogen receptor-related receptor1 ERR1, and AhR) was measured in the pituitary, the uterus and the

thyroid. Both the ERR1 and the AhR are known to modulate the ER's signalling pathways in multiple ways.

A significant increase was observed in uterine weight in the case of BP-2 treated groups, while the BP-3 treatment groups showed no effect.

In the pituitary, no effect in the expression of ERR1 mRNA was measured and BP-3 also had no effect on ERβ mRNA level. AhR mRNA expression was significantly decreased at high doses of BP-3 - an effect not seen in the E2-treated animals. Hence, it most likely acted *via* non-ER-mediated mechanisms. The strongest effect on the ERα gene expression (decrease in expression) was seen in both BP-3 treatment groups. This downregulating effect of BP-3 on ERα mRNA in the pituitary gland suggests that it has effects in estrogen-receptive organs which are not yet known.

In the uterus, BP-3 did not alter uterine ERR1 mRNA levels. A decrease in AhR mRNA expression was observed but not considered significant. ERβ gene expression was decreased by low dosage of BP-3. Hydroxylated benzophenones are known to exert E2-like effects in the uterus, i.e. to stimulate uterine weight. However, BP-3 was devoid of any uterotrophic effect and would therefore not be classified as an endocrine disruptor following the OECD protocol. This is in contrast to data published by Schlumpf et al. (2001), but that study had observed effects only in immature rats at high doses (1500 mg/kg/day).

In the thyroid, low dosage of BP-3 did not exert an effect on AhR expression. The expression of ERR1 mRNA was decreased slightly but significantly in high dosage treatment groups of BP-3. No clear effect of on the expression of ERα mRNA was reported, while ERR1 gene expression was downregulated moderately. The effects mediated via ERR1 in the thyroid gland are unknown at present.

The study concluded that BP-3 was devoid of any uterotrophic effect but inhibited ERα transcript expression and also mildly inhibited ERβ gene expression in the uterus. The study concluded that BP-3 exerts effects which are not covered by the uterotrophic assay, but might prove to be adverse. Effects mediated via AhR and ERR1 in these organs are unknown, so the physiological relevance of the findings remains to be established. In brief, unlike BP2, an estrogen-like effect was not observed for BP-3, although regulatory effects on receptor expression were observed indicating potential endocrine disruption that is not assessed by 'classical' estrogenic markers.

BP-3 did not show any induction of proliferation in MCF-7 breast cancer cells and estrogen-responsive CHO cells at concentrations of the order of micromolar (Watanabe *et al.* 2015; Kerdivel *et al.,* 2013). However, studies of BP-3 metabolism in rats have revealed that BP-3-can be converted into at least three metabolites that exhibit estrogenic potencies, BP1, BP8 and THB [Nakagawa, 2002; Jeon et al., 2008].

In a recent study, Alamer and Darbre (2018), have investigated the effects of Benzophenone BP-1, and other UV-filters on migration and invasion of human breast cancer cell lines. Their results have shown that long-term exposure to the UV filters BP-3 at 10^{-7} M following 21 weeks exposure can increase the migratory and invasive properties of two human breast cancer cell lines *in vitro.* Increased migration was observed following exposure to these UV filters in both oestrogen responsive (MCF-7) and oestrogen unresponsive (MDA-MB-231) human breast cancer cells, implying that their ability to increase cell motility is not confined to oestrogen-responsive cells. However, despite their oestrogenic activity in gene expression assays, BP-3 was not able to increase MCF-7 proliferation.

Majhi et al. (2020) investigated the estrogenic effects of BP-3 *in vitro* in different cell lines, and in ovariectomized BALB/c mice administered orally with BP-3. The results showed that in T47D cells, treatment with BP-3 at 1 μM or 5 μM increased γ-H2AX intensity in comparison

with the control (p <0.0001), but no dose-dependent increase was observed (1µM vs 5 µM BP-3).

In MCF-7 cells, a dose-dependent increase in nuclear γ-H2AX intensity was observed after treatment with BP-3 in the range of 1–30 µM. BP-3 treatment (1–5 µM) showed increased nuclear 53BP1 intensity over control in both T47D and MCF-7, but only MCF-7 showed dose-dependent increase. The effect on γ-H2AX was however contrasted with the mRNA expression of estrogen-responsive gene TFF1, as responses to 5 µM BP-3 did not differ significantly from the control. In comparison, treatment with 10 nM E2 stimulated a 13.1-fold increase in the expression of TFF1. Based on the measures of transcriptional activity in MCF7 human breast cancer cell lines, the authors concluded that typical exposures to BP-3 appear to pose a minimal risk for breast cancer through ER-mediated transcriptional activation of target genes.

In T47DKBluc cells, which harbour an integrated ERE-luciferase reporter, BP-3 showed a lowest-observed-effect at 5 µM with transactivation increasing to a maximum 37% relative transactivation activity (RTA) in comparison with 10 nM E2.

In T47D and MCF-7 cell lines, the expression of endogenous ER target genes - AREG and PGR - was also quantified. Treatment with BP-3 at 1 µM resulted in no significant changes in mRNA expression of AREG and PGR, a concentration that led to significant increases in DNA damage in both T47D and MCF7 cells.

In T47D cells, proliferation induced by BP-3 was also compared with control treatment. BP-3 had only marginal effect at 5 or 50 µM. Low concentrations of BP-3 only marginally increased cell numbers in comparison with control.

In Silico Modelling

An *in silico* modelling study by Hong et al. (2016) used consensus classification modelling that classified substances into ER binders or non-binders based on two Decision Forest (DF) models built using two different training datasets. The DF models were validated using five-fold cross validations and external chemicals but did not take into account of possible metabolic activation/deactivation of the compounds. The models predicted BP-3 as ER non-binder.

In contradiction, Sosnovcová et al. (2016) reported *in silico* assessment using QSAR Toolbox to predict more than 900 compounds from the list of food contact materials for potential to bind on ER. For the majority of the chemicals, the presence of a charge in the molecule, absence of hydrogen bonding group or inappropriate geometry could explain the lack of binding to ER. The features identified for the chemicals with high ER binding activity included two oxygen atoms available for hydrogen bonding at the specific distances dictated by the ER receptor. The study also considered molecular weight and partition coefficient of the evaluated compounds and predicted BP-3 to have a strong binding affinity for the ER binding site.

- <u>(anti)-Androgenic Disrupting Effects</u>

BP-3 showed no agonistic activity towards AR (Ma et al., 2003); however, it exhibited anti-androgenic activity in various cells-based bioassays (Schreurs et al., 2004; Suzuki et al., 2005; Kunz and Fent, 2006; Molina-Molina et al., 2008).

<u>Other endocrine MoA</u>

BP-3 also exhibited disrupting effects towards progesterone (PR) (Schreurs et al., 2004). Although BP-3 did not activate PR, it is the antagonist of PR as revealed by PR CALUX1 bioassay. As evaluated by an *in vitro* reporter system containing a duplicated thyroid hormone response element of the HLA-DR4 serotype, BP-3 can induce luciferase activity, showing agonistic activity toward THR (Schmutzler et al., 2007).

HMB was evaluated in quantitative (dose-response) high throughput screening assays by NTP in the Toxicology in the 21st Century (Tox21) program, and significant activity was observed in assays measuring stimulation of ER, progesterone receptor, constitutive androstane receptor, pregnane X receptor, retinoic acid receptor, and estrogen-related receptor signaling pathways (NTP report, 2020).

3.4.9.2 Endocrine-related Adverse Effects

Human data

According to the review by Siller et al. (2018), statistically significant associations between BP-3 exposure during pregnancy and varying birth outcomes have been reported in various studies. One study reported shorter pregnancy length in women gestating male fetuses, two studies reported higher birth weights in male neonates, and one found lower birth weights in female neonates (Ghazipura et al., 2017).

The effect of 3 UV filters on the hypothalamic- pituitary-thyroid axis in humans after whole body topical application was investigated in a 2-week single-blinded study. In this study 15 young men and 17 postmenopausal women were assigned to daily whole-body topical application (2 mg/cm²) of a basic cream in week 1 and with a mixture of the three sunscreens each at 10% (w/w) including BP-3 in week 2. The daily mean amount of cream applied over 4 days for each subject was 40 g for men and 35 g for women. All subjects were healthy and not taking any regular medication. The thyroid and postmenopausal status was verified about 4 weeks prior to treatment and hormone levels were measured. There was no biologically significant effect on hormone levels (TGB, TSH, T4 total, T4 free, T3 total, T3 free), indicating that the concentrations of sunscreen compounds absorbed were not capable of disturbing the homeostasis of thyroid hormones in humans.

(Janjua et al., 2007 from SCCP/1201/08)

Animal data

A few *in vivo* studies have recently investigated ED-related effects of BP-3. These include Nakamura et al., 2015; Laplante et al., 2018; Matouskova et al. 2019; Majhi et al., 2020 and Santamaria et al., 2020.

Nakamura et al. (2015) determined the effects of maternal and lactational exposure to BP-3 on development and reproductive organs of offspring of time-mated female Harlan Sprague-Dawley rats (11- to 13-week-old; seven to eight per group). Pregnant dams were housed individually and maintained under a 12 hr/12 hr light/dark cycle with controlled room temperature (23 ± 3°C) and humidity (50 ± 20%). Starting from gestation day 6 until weaning on postnatal day (PND) 23, dams were fed low-phytoestrogen chow containing 0, 1000, 3000, 10,000, 25,000, or 50,000 ppm BP-3. From the amounts of feed consumed, the authors calculated average consumed doses as 0, 67.9, 207.1, 670.8, 1798.3, and 3448.2 mg/kg/day respectively.

There was no significant difference in the number of implantation sites/litter, mean resorptions/litter, % litters with resorptions, number and weights of live foetuses, or sex ratios between the control and treated groups. In general, exposure to <10,000 ppm BP-3 was not associated with adverse effects on the reproductive system in rats. Reduced organ (liver, kidney) weights were in female and male offspring and blood biochemical factors in the high dose (≥10,000 ppm) groups.

The effects noted included a decrease in body weights of dams at GD 10, 15, and 20 in a dose-dependent manner with a significant difference apparent at 50,000 ppm as early as GD

10. In dams, at 50,000 ppm, at PND 23, BP-3 caused toxicity on the liver system as indicated by an increase in the weight of the liver, ALT, ALP and total bile acids.

Other effects observed in the highest dose groups included a decrease in the normalised anogenital distance in male pups at PND 23; impairment of spermatocyte development in testes of male offspring and delayed follicular development in females.

Although testosterone levels in PND 23 male rat offspring were lower in the groups exposed to 3000 and 25,000 ppm BP-3 compared to the controls, these decreases were considered sporadic and do not appear to be treatment related.

The authors concluded that the doses at which the adverse effects were observed were much higher than usual human exposure levels.

SCCS comments

This study determined the effects of maternal and lactational exposure to BP-3 on the development and reproductive organs of the offspring of time-mated female rats. The concentration of BP-3 fed through chow was 0, 1000, 3000, 10,000, 25,000, or 50,000 ppm (equivalent to average consumed doses of 0, 67.9, 207.1, 670.8, 1798.3, and 3448.2 mg/kg/day respectively).

The study did not find any significant differences on the reproductive/ pregnancy parameters or sex ratio of the offspring between the control and BP-3 dosed groups. The observed effects were mostly in the highest dose groups. These included a significant reduction in the normalised AGD in the highest (50,000 ppm) dose group of PND 23 male pups, a decrease in serum ALT and a significant increase in cholesterol levels in both female and male offspring at PND 23 in the 25,000 and 50,000 ppm dose groups. Body, ovarian and uterine weights were significantly decreased in the 25,000 and 50,000 ppm dose groups. Relative liver to body weight ratios were also significantly increased and paired kidney weights were significantly lower at 50,000 ppm dose group.

Also, observations on testis morphology of PDN 23 rat offspring showed that seminiferous tubules contained few or no spermatocytes compared to control group in the 50,000 ppm dose group. The number of spermatocytes per seminiferous tubule was significantly reduced at doses of 3,000 ppm and higher.

The authors considered that decrease testosterone levels observed at 3000 and 25 000 pm is not related to BP-3.

SCCS considers that it would have been useful to have measured foetal testosterone as it is known that a decrease during specific windows of sensitivity in male foetuses mat impaired the development of reproductive system (↓ AGD, ↓ spermatocytes production and/or quality…).

For counting the number of spermatocytes per seminiferous tubule, a total of 40 seminiferous tubules from one or two male pups from each of 3 or 4 litters/group were selected at random, based on the round shape of seminiferous tubules and stage VII-IX of spermatogenesis, which is an appropriate approach.

Apoptosis is also increased in the tubules (see Fig 6); it is known that germinal cells are more sensitive than Sertoli cells to apoptosis (and Sertoli cells are less in numbers in the tubules).

Moreover, it is well known that testosterone is needed for meiosis (De Gendt et al., 2004). Therefore, a decrease in testosterone, which is significant at 3000 ppm (see Fig 7) supports the plausibility of a decrease in spermatocytes numbers.

Due to the effects on the ↓ of spermatocytes, the SCCS considered 3000 ppm as a LOAEL and therefore the NOAEL is 1000 ppm (67.9 mg /kg bw/day). This value will be used by the SCCS as a point of departure (POD) for the MoS calculation (see section 3.5 safety evaluation)

Laplante et al. (2018) compared BP-3 exposed female mice with vehicle-exposed parous (i.e. that had given birth) and nulliparous (i.e. that had not given birth) female mice to evaluate potential interference in the reorganisation of the mammary gland that is characteristic of pregnancy, lactation and involution.

The study is divided in two parts: an acute exposure study and a chronic study in which animals were exposed during pregnancy, lactation and post-involution.

- In the **acute exposure study**, 6 to 8-week old female Balb/C ovariectomised mice were exposed orally during 4 days to BP-3 (3000 µg/kg/d, n=6), or 17β-estradiol (250 µg/kg/d, n=4) as a positive control, or vehicle as a negative group (n=6). One day after the exposure, the mice were euthanised and uteri weighted.
- In the **chronic exposure study,** 8-week old female Balb/C mice housed in polysulfone cages with food (which may have contained phytoestrogens) were exposed orally to BP-3 (>99% purity) from Gestational Day (GD) until the day before weaning (Lactational Day 21 or LD21) at 3 doses of BP-3 (30, 212 and 3000 µg/kg/d).

The lowest dose (30 µg/kg/d) was chosen on the basis of equivalence to the EFSA Tolerable Daily Intake (TDI), whereas 212 µg/kg/d for being close to the 95 percentile reported in human biomonitoring studies in pregnant women, and 3000 µg/kg/d for equivalence to the NOAEL used by EFSA as a point of departure (POD) to derive the TDI. The authors noted that doses around 10 times higher are needed in rodents to produce equivalent levels in human urine, which means that the highest dose was still environmentally relevant.

The study included 2 negative control groups: 1 vehicle group and a second group of nulliparous female mice, which were housed under the same conditions.

At 5 weeks post-weaning (once mammary gland involution was complete), mice were killed and mammary glands (the left and right third) collected and prepared for further analysis: whole-amount (n =12-14), and histological and immunohistochemical examinations (n=5-7).

The expression of four markers (ER-alpha, Ki67, PR, Wnt5a) was also evaluated in the study.

The results of the acute exposure study showed that BP-3 (3000 µg/kg/d) did not have any effect on the uterus weight. It did however produce significant but modest increase in expression of ERα.

In the chronic study, BP-3 did not show effects on the number of pups, and induced only modest non-statistically significant changes to the length of gestation.

The whole-amount mammary gland examination showed that at 3000 and 30 µg/kg/d, BP-3 did not show any effect on the ductal density compared to the vehicle-exposed group. However, at 212 µg/kg/d, females had mammary glands with significantly lower ductal densities than the vehicle group - consistent with an intermediate phenotype between nulliparous and parous mammary glands. Histological examination of the mammary gland showed that mammary glands of mice treated with BP-3 at 212 mg/kg /d or 3000 mg/kg/d had significantly more epithelium than the nulliparous gland. The mammary glands of mice treated with BP-3 at the dose of 30 mg/kg/d were not significantly different from either the nulliparous or the vehicle parous mammary glands - consistent with an intermediate phenotype.

- Quantification of the expression of Ki67 showed a dose-dependent increase in the BP-3 treated females, with a statistically significant increase at the dose of 3000 mg/kg/d compared with the vehicle parous females.
- Expression of Esr 1 in mammary epithelium - the gene encoding for ER alpha - in all three BP-3 treated groups was indistinguishable from both the nulliparous and the parous vehicle-exposed mice. Furthermore, the percentage of epithelial cells expressing ER alpha was not affected in any BP-3 treated group.
- PR mRNA expression was not affected by either BP-3 or parity. However, analysis of PR protein expression revealed an effect of both parity and BP-3 on PR-positive

epithelial cells. At the dose of 30 µg/kg/d, a decreased percentage of PR-positive cells was observed, but not at the 2 other doses.

- The study also found that BP-3 does not affect ER beta-mediated gene expression.

The authors considered that this study showed that BP-3 may alter mammary gland morphology and histomorphology. They concluded that substantial reduction in the ductal density in female mice exposed at 3000 µg/kg/d BP-3 produced an intermediate phenotype resembling a mix between the nulliparous and vehicle-parous mammary glands. They however noted the need for additional studies to determine if this intermediate phenotype is a result of BP-3 induced reorganisation of the mammary gland in pregnancy (e.g. limited proliferation), in lactation (e.g. incomplete differentiation of lobuloalveolar structures), or during the process of involution (e.g. excessive apoptosis and clearing of epithelial structures). They considered the results to show that the effects of exposure to BP-3 during pregnancy and lactation can have long–lasting effects on the morphology of the mammary gland as the direct target of BP-3 exposure, or as results of long term alterations to the hypothalamic-pituitary-ovarian axis.

Matouskova et al. (2019) studied the endocrine-disrupting potential of BP-3 on male and female BALB/c mice exposed during gestation and perinatal periods.

Test animals used in this study were the same as in Laplante et al., 2018 study; i.e. 6-8-week old BALB/c female mice in 4 treatment groups, and offspring evaluated at 3 life stages - prior to puberty, at puberty, and in adulthood. Female mice were mated and housed in polysulfone cages. After weaning, offspring were housed under the same conditions, 4–5 same-sex (and same-treatment) animals per cage.

The doses of BP-3 used were 30 µg/kg/day, 212 µg/kg/day, and 3000 µg/kg/day. Female mice were orally dosed daily by pipet with either tocopherol-stripped corn oil or BP-3 dissolved in tocopherol-stripped corn oil from the pregnancy day-zero until the day before weaning (on lactational day-21). Thus, the offspring evaluated were exposed *in utero*, and during the first 21 days of postnatal life via the mother.

Treatment groups consisted of controls (11 litters); 30 µg/kg BP-3 (10 litters); 212 µg/kg BP-3 (11 litters); 3000 µg/kg BP-3 (9 litters).

The study calculated anogenital index (AGI) as the anogenital distance divided by body weight to account for differences in body size. From every male animal, both the left and right fourth inguinal mammary glands were collected. From every female animal the right fourth inguinal mammary gland was dissected. The left mammary gland was fixed in 10% neutral buffered formalin overnight before washing, dehydrating, and embedding in paraffin for histology and immunohistochemistry. The whole mounted mammary glands were imaged with a Zeiss AxioImager dissection microscope.

The expression of 3 markers was evaluated using standard methods for immunohistochemistry and commercial antibodies. These included rabbit anti-ERα; rabbit anti-Ki67 (a marker of proliferation); and rabbit anti-progesterone receptor.

The results showed no effect of BP-3 in males on the body weight at any age, even when litter size was treated as a covariate to control for the differences in growth parameters. The AGI was reduced after exposures to 30 µg and 212 µg doses ($p<0.05$) at postnatal day 21 and in puberty. However, in adult males, no differences in AGI were observed. At postnatal day 21, developmental exposure to BP-3 significantly reduced the size of the mammary gland epithelium in males on both the left and right sides ($p<0.05$). At puberty, there were no statistically significant effects of BP-3.

There were no statistically significant differences after BP-3 exposure in the mammary gland morphology in males in early adulthood. At postnatal day 21, the mammary glands from all three male treatment groups had significantly fewer branching points compared to controls

(p<0.05). However, by puberty and in adulthood, the control glands were less complex in their branching, and there were no longer significant differences based on BP-3 treatment.

In females, developmental exposure to BP-3 did not affect body weight at postnatal day 21. However, at puberty, when litter size was treated as a covariate, females exposed to 3000 µg/kg/day BP-3 were heavier compared to controls (p<0.05). Furthermore, females exposed to 212 µg/ kg/day BP-3 were also heavier in adulthood compared to controls (p<0.05). The AGI in females was unaffected at postnatal day 21, but decreased by exposure to 212 µg/kg/day when measured at puberty (p<0.05). No effects of developmental BP-3 exposure were observed on female AGI in adulthood.

At postnatal day 21, the whole mounted mammary glands from females exposed to the highest dose of BP-3 had modestly larger ductal areas compared to controls (p<0.05) but no significant effects were observed for the extent of growth into the fat pad (ductal extension). Terminal end buds (TEBs) were observed in a few animals, with no significant differences in number or size based on BP-3 treatment. At puberty, the females exposed to 30 and 3000 µg BP-3/kg/ day had a modest increase in the size of the ductal tree and a significant increase in the extent of growth into the mammary fat pad. There was also a significant increase in the number of TEBs in females exposed to 3000 µg BP-3/kg/day (p<0.05), as well as a trend toward significance for a linear increase in number of TEBs by BP-3 dose (Pearson correlation 0.324, p=0.058). There were no significant differences in total TEB area between BP-3 treated groups and controls although there was a trend toward significance for a linear increase in TEB area by BP-3 dose (Pearson correlation 0.304, p=0.075)

Analysis of adult mammary glands showed a decreased fraction of the mammary gland comprised of ducts as the dose of BP-3 increased. The opposite pattern was observed for the fraction of the gland comprised of alveolar buds, with a significant increase observed in females from the 3000 µg BP-3/kg/day group (on average, >20 times more alveolar buds compared to controls). There was also a trend toward significant linear increase in volume fraction of alveolar buds by BP-3 dose (Pearson correlation −0.306, p=0.065). There were no significant differences in volume fraction of terminal ends based on treatment.

A decrease in the fraction of cells positive for ERα in the mammary gland of females exposed to BP-3 was observed. The decreased fraction of ERα-positive cells was however only significant in adult females after developmental exposures to 30 or 212 µg/kg/day (p<0.05). No significant effects were observed on fraction of cells expressing progesterone receptor in mammary glands at postnatal day 21 or in adulthood, but a significant decrease in progesterone receptor-positive epithelial cells was observed at puberty in females exposed to 212 µg BP-3/kg/ day.

Investigations into the expression of Ki67, a marker of cell proliferation in mammary gland epithelium, showed that developmental exposure to BP-3 reduced the fraction of proliferating cells, although only the females from the 30 µg/kg/day group were significantly different from controls at postnatal day 21. There was a significant linear relationship between BP-3 dose and fraction of cells positive for Ki67 (Pearson correlation 0.447, p = 0.006). In adulthood, proliferation in the mammary epithelium was not affected by developmental BP-3 exposure.

From this study, the authors concluded that exposures to low doses of BP-3 altered AGI in both males and females, decreased the size and complexity of the mammary ductal epithelium in males, and increased the appearance of alveolar buds in adult females. BP-3 altered the expression of hormone receptors in female mammary gland in an age- and dose-specific manner, and disrupted proliferation in the mammary epithelium.

BP-3 appeared to affect young males more than females, although the effects observed on the adult female mammary morphology (e.g., increases in alveolar buds) were remarkable. The study also showed more effects induced by 30 and 212 µg BP-3/kg/day than the higher dose of 3000 µg/kg. The two lower doses were considered by the authors as relevant to human exposures based on back-calculations of urinary concentrations in pregnant women.

While recognising that it is difficult to draw definitive conclusions about dose-response relationship on the basis of only three treated groups, the authors considered that the results

may be consistent with nonmonotonic effects of many endocrine disrupting chemicals. They suggest that the findings are consistent with what would be expected if BP-3 was a hormonally active endocrine disruptor.

SCCS comments on Laplante et al. (2018) and Matouskova et al. (2019):
The two studies by LaPlante et al. (2018) and Matouskova et al. (2019) are from the same laboratory and have used the same doses 0, 30, 212, or 3000 µg BP-3/kg/d for oral dosing of 6-8-week old female mice daily from pregnancy day 0 until weaning.
Both papers have measured a number of parameters to investigate estrogenic activity of BP-3, even though it is reported that it does not appear to be an agonist for ERβ, and the suspected estrogenic activity has been mainly attributed to its metabolites, and that BP-3 is also antiestrogenic and antiandrogenic in some contexts.

The doses used in these studies are far below those used by the National Toxicology Program where 2 years exposure to BP-3 (that used doses ranging from 15 to 65 mg/kg/d), showed decreased incidence of fibroadenomas in the female F344/N rat, possibly as a result of the inhibition of steroid sulfatase (which regulates the formation of estrone and its conversion to estradiol).

The acute study carried out by LaPlante et al. (2018) is equivalent to the uterotrophic assay OECD 440. The content of phytoestrogens in the diet used in the study and whether it could have influenced the results was not reported. Other effects reported in the chronic study at lower doses (212 µg/kg/d or 30 µg/kg/d) are either statistically non-significant or a result of modification in the expression of certain genes. It is also not clear whether or not the observed effects on the mammary gland were reversible or whether these could lead to some adversity.

The SCCS has noted that the results presented in these studies are often in contradiction with the authors' conclusions in regard to dose response, non-monotonic dose response, estrogen and progesterone time of response etc, and do not demonstrate a clear correlation between the treatment and the measured parameters. Therefore, whilst the studies may indicate potential endocrine activity of BP-3, they do not provide any clear scientific evidence that may be used in safety assessment of BP-3.

Majhi et al. (2020) carried out an *in vivo* study in ovariectomised BALB/c mice, administered orally with BP-3 (3,000 µg/kg/d) and 17β-estradiol (250 µg/kg/d). The nuclear c-H2AX intensity in the mammary gland of both BP-3 and 17β-estradiol treated animals was noted to be significantly higher than the animals treated with vehicle control. However, whilst 17β-estradiol led to stimulated proliferation of mammary epithelium and increased uterine weight, no significant effects were observed for BP-3.

Santamaria et al., (2020) have investigated how BP-3 may have effect on the progeny of mice after dermal exposure during early pregnancy. As this study has been specifically performed to address concerns expressed by SCCS and also by the FDA due to the cosmetic uses of BP-3 as an UV filter, it is detailed below:

Guidelines/Guidances:	/
Test item:	Benzophenone 3 (BP-3)
Purity:	Not described
Vehicle:	Olive oil
Route of exposure:	dermal on the back
Duration of exposure:	Group 1: from GD0 to GD6 Group 2: from GD0 to GD6 Group 3: from GD0 to GD6
Doses:	50 mg/kg bw/d equivalent to calculated human dose after whole body application according to Janjua et al., 2018
Observations	Group 1: sacrificed at GD5, GD 10, GD14

	HF Ultrasonic Imaging at GD5, GD8, GD10, GD12 and GD14
Group 2:	sacrificed during second pregnancy at GD 10 and GD14
Group 3:	weights of the pups measured from PND1 to PND19 every 3 days after 2 pregnancies

Experimental animals:	
Species:	Mice
Strain:	Female C57BL/6J (and male BALB/c)
Sex:	Female
Animal numbers:	Group 1: treated mice: n = 19 (n = 4-6 for each time point); control: n = 19 (n = 4-6 for each time point) Group 2: treated mice: n = 9; control: n = 7 (n = 3-5 for each time point); Group 3: treated mice: n = 19; control: n = 19 (n = 4-5 for each time point);
GLP:	/

In this study, mice were dermally exposed to BP-3 at the dose of 50 mg/kg bw/day during early gestation. Critical parameters of pregnancy such as implantation rate, intrauterine embryonic and fetal development were investigated.

BP-3 (but not its metabolites) was also measured in blood samples at GD6 and in blood and amniotic fluid in GD14 during the first pregnancy and at GD10 and GD14 during the second pregnancy.

BP-3 was detected in serum 4 hours after the last dermal application (GD6): mean = 22.4 +/- 2.3 ng/mL (n = 3) whereas no BP-3 was measured in control mice. In only 1 mouse among 3, BP-3 was quantified in serum at GD14: mean = 16.8 ng/mL but in all mice in amniotic fluid mean = 22.63 +/- 10.8 ng/mL. No BP-3 was detected in serum of mice at GD10 or GD14 of the second pregnancy.

Concerning reproductive parameters, the results show that:

- After the first pregnancy:
  - The areas of the implantation measured at GD5, GD8, GD10 and GD12 showed no differences between BP-3 treated and control mice: no effects on the number of implantations, abortions, and abortion rates were reported.
  - Placental parameters such as placental area, diameter, thickness at GD10 and GD12 were not different between groups
  - Fetal weight in BP-3 treated mice was significantly reduced at GD14 compared to control mice and placental weight was not modified, therefore the feto-placental index was reduced. Moreover 16.13% of foetuses in the BP-3 group were under the 5^{th} percentile meaning that there are affected by intrauterine growth restriction.
  - Hemodynamic parameters of the maternal arteria uterine showed altered pattern at GD10 but not at other time point (GD5, GD8, GD10, GD12 and GD14) and at GD10 no alterations of the uterine spinal arteries were reported.
  - Based on the weight curve of the progeny, it can be observed that male offspring from exposed mice were lighter at PND4 and then from PND10 onwards. On the other hand, females born to BP-3 exposed mice showed decreased weight at PND1, PND10 and PND13, but then recovered their normal weight from PND16 forward. Thus, decreased weight persist until weaning only in males.
  - The sex ratio was modified with more females than males in the offspring of BP-3 treated mice (84% versus 64% in control group).

- After the second pregnancy:

  - No effect were reported on implantations and abortion numbers
  - The placenta weights from BP-3 treated mice was significantly reduced at GD14 but not at GD10. Fetal weight and fetus/placenta index showed no difference between controls and BP-3 treated mice.
  - No modification of the weight curves of the offspring were reported
  - The sex ratio was modified with more females than males in the offspring of BP-3 treated mice

Conclusion from the authors
The results suggest that dermal exposure to BP-3 at a low internal dose during early pregnancy could impair fetal development, resulting in growth-restricted fetuses. The intrauterine growth restriction (IUGR) phenotype of the fetuses was associated with transient changes in hemodynamic parameters at maternal uterine artery, reduced weight of male offspring and persistence of significant levels of BP-3 in amniotic fluid until GD14.
A very interesting observation done in our studies was an alteration of the sex ratio of the progeny, with females predominating over males when analyzing both pregnancies.

SCCS Comments
The SCCS sought clarification on a few aspects of the study from the authors of Santamaria et al. (2020). Their response indicated that:

1. A combination of two different strains of mice (C57BL/6J female and BALB/c male) was considered the most representative setting of human pregnancy representing an allogeneic combination of mice rather than syngeneic one. This was also regarded as important from immunological point of view.
2. BP-3 used in the study was 98% pure (Sigma, catalogue H36206).
3. The study was performed in Germany and Argentina as part of a cooperation project. The same vehicle (extra virgin olive oil) was used for treatment of control and BP-3 treated groups of mice.
4. Oral exposure of mice through grooming was prevented during the study by applying the treatment on shaved area of animal's back during the first 6 days of gestation. The treatment was also applied to pregnant mothers, and each of them was housed in an individual cage from beginning of pregnancy.
5. The concentration of phytoestrogens in the diet was not evaluated, but it was assumed that both treated and control groups were exposed to the same levels of phytoestrogens as the food intake was equivalent in both groups.
6. The potential exposure to endocrine disrupting chemicals from cages and litter was minimised through housing the mice in stainless steel cages with sterile pine wood shavings as bedding. In addition, glass bottles with rubber stoppers for the supply of drinking water had stainless steel stops, which prevented the animals from touching the rubber material of the stopper.
7. Blood samples were drawn from mice via cardiac puncture because it was not known how much volume would be needed for reliable analysis of BP-3, and this method was chosen because it would yield the highest volume of blood.
8. Ultra-high-performance liquid chromatography was used to measure only BP-3 in blood. No metabolites were measured because high purity standards for BP-3 metabolites were not available.
9. Intrauterine embryonic and fetal development was examined using ultrasound method (Vevo 2100 System - Fuji Film Visualsonics Inc.), using the protocol described in a previous publication (Meyer, N., Schüler, T., Zenclussen, A. C. High Frequency Ultrasound for the Analysis of Fetal and Placental Development In Vivo. J. Vis. Exp.

(141), e58616, doi: 10.3791/58616, 2018).

10. The difference in sex ratio was derived from both progenies. It was acknowledged that the number of animals specified in the legend of Figure 6 needed correcting. The authors hypothesised that observed effects on the progeny after the second pregnancy, where foetuses were not exposed to BP-3, might have been due to: 1) epigenetic alterations that altered placental growth, and 2) the alteration of sex ratio might be causally related to hormone levels of both parents around the time of conception. However, hormonal levels were not measured in this study.

Taking into account the information from the article, and further clarifications provided by the authors upon SCCS request, the SCCS is of the view that the study is of sufficient quality and the protocol used is well adapted to investigate the potential effects of BP-3 following dermal exposure. The notable effects reported in this study include:

- A decrease in fetal weight and feto-placental index but not placental weights on GD14 in 1st pregnancy. No significant effects observed during 2nd pregnancy. The magnitude of these effects is limited.
- Any significant differences between BP-3 treated and control groups in terms of blood velocity parameters of the maternal arteria uterina (UA) were only observed on GD-10 and not on other days (GD5, GD8, GD12 and GD14). These included end diastolic velocity (in control mice), and systolic/diastolic ratio, resistance index and pulsatility index in treated mice.
- A decrease in the weight of offspring in the BP-3-treated group in 1st pregnancy - from the middle of lactation until weaning in male and female pups, and after birth. The magnitude of these effects is limited.
- Change in sex ratio of the offspring in BP-3-treated group. Females (sex ratio) was higher in both in the first and second pregnancies of mothers (that were exposed to BP-3 only during GD0 to GD6 of the first pregnancy), compared to the vehicle-treated controls. The percentage of males and females of the first and second pregnancies have been put together in one Figure – Fig. 6. Having seen the individual data provided by the authors upon SCCS request, the SCCS is of the view that this finding has limited statistical significance because the number of litters is too low to make any robust conclusion. Only few other publications have investigated alterations in sex ratio following the exposure of test animals to chemicals. In a review of such effects following TCDD exposure, Rowlands et al. (2006) concluded that the inconsistency in findings on the sex ratio of the offspring of male rats exposed to TCDD *in utero* is likely due to random variation associated with a relatively small sample size, although differences between studies relating to strain of rat, dose regimen, and day of ascertainment of sex ratio could not be ruled out.

In conclusion, the SCCS considers that whilst this study indicates a potential reproductive effects of BP-in mice, the observations need to be consolidated through further research, and for the time being it does not provide any clear scientific evidence that can be used in the safety assessment of BP-3.

SCCS Overall Conclusions on the ED Properties of BP-3

The currently available evidence for endocrine disrupting properties of BP-3 is not conclusive, and is at best equivocal. This applies to the data derived from *in silico* modelling, *in vitro* tests and *in vivo* studies, when considered individually or taken together. There are either contradictory results from different studies, or the reported data do not show dose-response relationship, and/or the effect are seen only at relatively very high doses that can only be considered far beyond the human exposure range. In view of this, the SCCS considers that

whilst there are indications from some studies to suggest that BP-3 may have endocrine effects, it is not conclusive enough at present to enable deriving a new endocrine-related toxicological point of departure for use in safety assessment.

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

The SCCS has used the lowest NOAEL values among those obtained from different toxicological endpoints for the calculation of MoS (summarised in Annex-1). The NOAEL of 67.9 mg/kg bw/d has been derived from Nakamura et al. (2015) study. Because of the evidence for rapid and almost complete absorption of BP-3 from the oral route, the SCCS has not applied any adjustment for bioavailability to this NOAEL value. Details of the calculation of systemic exposure dose (SED) are presented in the Tables in Annex-2. The calculation of MoS for different product types are given in Table 3 below:

Table 3: Calculation of Margin of Safety for different products containing BP-3

Products	Conc.	Surface	Systemic Exposure Dose (SED) mg/kg bw/d				NOAEL*	MOS
			Dermal	Inhalation	Oral	Total		
UV cream	6%	whole body	1.782	0	0	1.782	67.9	38
UV aerosolised spray	6%	whole body	1.782	0.105	0	1.887	67.9	36
UV pump spray	6%	whole body	1.782	0.001053	0	1.783	67.9	38
UV face cream	6%	face	0.152	0	0	0.152	67.9	447
UV hand cream	6%	hand	0.214	0	0	0.214	67.9	317
Non-UV**	0.50%	whole body	0.116	0	0	0.116	67.9	585
Lipstick	6%	lips	0.000	0	0.054	0.054	67.9	1257

* derived from prenatal and postnatal developmental study, oral, rat (Nakamura et al., 2015)
** to protect cosmetic formulations

Consideration of BP-3 exposure data in humans

The Nakamura et al. (2015) study, used as the key study in this evaluation for the calculation for MoS, measured the internal dose of BP-3 and metabolites in the serum of dams at GD 10, 15, 20 and PND 23. Serum levels of HMB and 2,4-dihydroxybenzophenone (DHB), 2,3,4-trihydroxybenzophenone (THB) and 2,2-dihydroxy-4-methoxybenzophenone (DHMB)] were measured using HPLC tandem mass spectrometry. HMB and DHB levels in the dose groups increased in a dose-dependent manner at GD 10, 15, 20 and PND 23. THB and DHMB metabolite levels were not detected in this study (see Table 3) .

This can be put into perspective with the human exposure by comparing with a human study (Janjua et al., 2008) that evaluated the plasma concentration of BP-3 at 0 to 96h after application of BP-3 (10%) in cream (2 mg/cm2 corresponding to 40 g/ 2 m2 of body area) on human skin daily for 4 days. The peak levels of BP-3 (median) in plasma after 3-4h treatment

were 0. 187 µg/ml in females and 0.238 µg/mL in males. Twenty-four hours after the first application, the median BP-3 levels in plasma were 0.047 µg/ml in females and 0.041 µg/ml in males. This clearly shows that the levels of BP-3 had exceeded the concentration measured in rats at the NOAEL.

3.6 DISCUSSION

The 2008 SCCP Opinion (SCCP/1201/08) had noted a number of shortcomings in the data provided as part of the dossier assessed in an earlier Opinion (SCCP/1069/06). Despite this, the SCS agrees with the SCCP Opinion (2008) that the submission provides a reasonably well-structured overview of the available test descriptions and publications.

Physicochemical properties

BP-3is a widely used UV filter in cosmetic products. It is an off-white to light-yellow powder with a melting point of 62°C to 65°C. It is relatively insoluble in water and is readily soluble in most organic solvents. It absorbs ultraviolet (UV) A (320 to 400 nm) and UVB (290 to 320 nm) light and is photostable.

Toxicokinetics

The SCCS has accepted the *in vitro* study used in the previous SCCP Opinion (SCCP, 2008) to determine the dermal absorption. The study has used the most conservative methodology followed by the use of only the values that had a good recovery. As there were a lot of samples at the start, enough samples were still available with a good recovery. Since the compound has a low solubility in PBS, ethanol (20%) was added to the receptor fluid, which is a procedure foreseen in the OECD protocol. As the protocol had to be changed (washing procedure, number of samples used), 2x SD were taken into consideration in line with the SCCS Notes of Guidance. The most conservative dermal penetration value was then used for the 2% test as the 6% test, and twice the most conservative SD was added for each of the concentrations. As such, the dermal absorption values are sufficiently conservative, considering that the compound is likely to go through extensive metabolism in the skin *in vivo*. Therefore, the SCCS considers that based on the *in vitro skin* penetration study performed with 6% BP-3, which corresponds to the maximum requested concentration, a dermal absorption of 9.9% [mean (3.1%) + 2 SD (2*3.4%)] has been used for the calculation of the systemic exposure dose (SED) and the margin of safety (MoS) for sunscreen products.

For other cosmetic products, the SCCS has considered a dermal absorption of 8% [mean (4%) + 2 SD (2*2%)] based on the results of the study performed with 2% BP-3.

The SCCS also considers that BP-3 is well absorbed after oral exposure and therefore the SCCS has not corrected the oral POD used for the MoS calculation to take into account oral bioavailability.

Exposure

BP-3 is currently regulated as a UV-filter in sunscreen products at a concentration, up to 6% in ready for use preparations (Annex VI/4). It is also allowed at a concentration up to 0.5% to protect product formulation in all other cosmetic products (Annex VI/4). Besides cosmetic products and personal care products, BP-3 is also used in the following products: coating products, fillers, putties, plasters, modelling clay and finger paints.

There are other sources of BP-3 releases into the environment – e.g. from indoor use such as machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners, and outdoor use as a processing aid. Considering the

widespread use of BP-3, exposure may also occur from sources other than sunscreens or personal care products.

BP-3 has been found in detectable levels in human urine, serum and breast milk and has been hypothesised to be from sunscreen use. Despite the detected levels being low in human samples, a study has detected BP-3 in 97% of the tested urine samples, with mean concentration of 22.9 ng/ml (95th percentile of 1040 ng/ml) (Calafat et al., 2008). A human volunteer study by Matta et al. (2019) has also indicated that application of 3 commercially available sunscreens formulations containing BP-3 at concentrations between 4-6%, under maximal use conditions, resulted in systemic absorption and associated plasma concentrations of 169.3 -209.6 ng/mL. In another study, Matta et al. (2020) found the systemic absorption and associated plasma concentrations of BP-3 to be between 180.1-258.1 ng/mL for aerosol spray and lotion respectively. Santamaria et al. (2020) reported BP-3 levels in serum in mice 4 hours after the last dermal application (GD6) (mean = 22.4 +/- 2.3 ng/mL, n = 3), and in amniotic fluid (mean = 22.63 +/- 10.8 ng/mL, n=3) confirming that BP-3 can cross the placental barrier.

Some studies have reported higher levels of BP-3 and other benzophenones in human urine samples. The frequent detection of BP-3 (and its metabolite BP-1) in breast milk or placental tissues also implies potential transfer to fetus and breastfed infant.

Toxicological Evaluation

Irritation and corrosivity
BP-3 is not considered as being irritating to the skin and the eyes.

Skin sensitisation
The clinical data considered in the 2008 SCCP Opinion, in conjunction with the results from more recent studies and the outcome of pre-marketing human repeated insult testing of products containing BP-3, confirm the previous evaluation of the SCCP that BP-3 can cause photoallergic reactions. The European photo-patch test task force stated that BP-3 is the second most frequent photo contact allergen among the UV filters. A case of anaphylactic reaction to BP-3 has also been recently reported.

Acute toxicity

The 2006 SCCP Opinion (SCCP/1069/06) stated that the studies reporting the acute toxicity values in terms of LD50-oral-rat and LD50-dermal-rabbit were not performed according to appropriate guidelines and GLP practices.

Repeated dose toxicity

The 2008 SCCP Opinion (SCCP/1201/08) noted that the studies provided as part of the dossier assessed in the earlier Opinion (SCCP/1069/06) had indicated that the most frequently encountered adverse effects observed after repeated oral administration of BP-3 in rats and mice consisted of unspecific signs of systemic toxicity in the form of reduced food consumption and retarded body weight gain, together with effects on the identified target organs being the kidney and the liver. These effects were partly associated with changes in clinical chemistry. Very often the most susceptible parameter was the increase in liver weight, but without histopathological correlation.

A recent 14-week study (NTP report, 2020) has indicated that the mean body weight of the male rats receiving dietary BP-3 at 10,000 ppm was not significantly different from the control group, but the mean body weight of the 10,000 ppm females was significantly decreased and was approximately 87% that of the control group at 14 weeks. In males, the absolute and relative liver and right kidney weights were increased in the 10,000 ppm group compared to the control group. In females, the absolute kidney weight was significantly decreased, and

the relative liver weight was significantly increased relative to the control group. Other changes in organ weights were not considered toxicologically relevant. Transcriptome analysis of the male rat livers from the 10,000 ppm and control groups was consistent with a mild induction of xenobiotic metabolism-related processes that is likely related to the observed relative liver weight increase. Analysis of a subset of estrogen-responsive genes showed no change in response to BP-3.

Reproductive toxicity

The 2008 SCCP Opinion (SCCP/1201/08) noted that the studies provided as part of the dossier assessed in the earlier Opinion (SCCP/1069/06) had indicated that:
'A teratogenicity study in rats showed BP-3 to be non-teratogenic under the conditions of the test. Only at the highest dosage level, which also caused maternal toxicity, some skeletal aberrations were noted. The NOAEL-value for maternal and developmental toxicity was 200 mg/kg bw/day. A NOAEL value of 400 mg/kg bw/day for reproductive toxicity was extracted from subchronic toxicity studies involving additional reproductive toxicity parameter measurements.'

A more recent NTP modified one-generation study found that gestation body weights of dams receiving 10,000 ppm BP-3 in the diet were slightly lower (~3%) than those of the control group and showed statistically significant differences. Dams receiving 3,000 or 10,000 ppm BP-3 in the diet displayed slight decreases in GD 6–21 body weight gain (~10%) relative to the control group that attained statistical significance. Lower body weight gain over the GD 6–9 (10,000 ppm) and 18–21 (3,000 and 10,000 ppm) intervals, which was associated with slightly lower feed consumption over the GD 18–21 interval, likely contributed to this response. These collective effects are minimal and would not be sufficient to affect normal development of the offspring.

Administration of BP-3 had no effects on the percentage of mated females producing pups, litter size, pup sex distribution, or numbers of male or female pups. The apparent decrease in the percentage of females pregnant in the 10,000 ppm group can be attributed to the seven animals that had no evidence of pregnancy (i.e., not exposure-related given that exposure began after implantation). Dams receiving BP-3 did not display any adverse clinical findings before or after parturition. Litter size of the 10,000 ppm BP-3 group was slightly lower on PNDs 7 and 10.

Male, female, and combined pup body weights were not significantly different across the exposure groups on PND 1. However, on PND 4, pup body weights (male, female, and both) in the 10,000 ppm exposure groups were ~10% lower than those of the control groups, and this response was generally observed over subsequent pre-weaning weights. Pups were weaned on PND 21, which was considered day 1 of the 2-year exposure period.

Mutagenicity / genotoxicity

The 2008 SCCP Opinion (SCCP/1201/08) noted that the studies provided as part of the dossier assessed in the earlier Opinion (SCCP/1069/06) had indicated that:
'As far as the (photo)mutagenic/(photo)genotoxic potential of BP-3 was concerned, the presented *in vitro* and *in vivo* assays indicated that the substance did not possess (photo)mutagenic of (photo) genotoxic properties.'

The National Toxicology Program (NTP) of the U.S. Department of Health and Human Services has published a draft technical report. Results of bacterial mutagenicity tests conducted using standard testing approaches with the same lot of BP-3 tested in the 2-year studies were negative in TA98 and TA100, as well as in Escherichia coli strain WP2 uvrA pKM101, with and without rat liver S9.

As noted in the SCCP/1201/08 Opinion, the newly provided data on gene mutations at the HPRT locus in V79 cells also support the safety of BP-3 in terms of genotoxicity.

Carcinogenicity

The National Toxicology Program (NTP) of the U.S. Department of Health and Human Services has published a draft technical report finding that ultraviolet ray-absorbing ingredient 2-hydroxy-4-methoxybenzophenone (HMB; BP-3; CAS 131-57-7) provides "equivocal evidence of carcinogenic activity" in rats. This level of evidence is the third level within five categories and is defined as representing "a marginal increase of neoplasms that may be chemical related."

BP-3 was nominated to NTP by the National Cancer Institute because of high exposure via use of BP-3-containing sunscreen products and lack of carcinogenicity data.

Two-year studies were completed in both mice and rats, exposing them to concentrations between 0 and 50,000 parts per million. It was found that exposure to BP-3 in rats led to an increase of non-neoplastic lesions of the testis and pancreas in males and of the uterus and adrenal cortex in females. Increases in lesions were also observed in the bone marrow, spleen, and kidney of both sexes in mice.

Special investigations: assessment of endocrine disrupting potential (including human data)

The SCCS has considered a number of recent studies into the potential endocrine effects of BP-3. Detailed analysis of these studies is provided in section 3.4.9. In brief, the SCCS considers the currently available evidence for endocrine disrupting properties of BP-3 as inconclusive and at best equivocal. This applies to the data derived from *in silico* modelling, *in vitro* tests and *in vivo* studies, when considered individually or taken together. There are either contradictory results from different studies, or the reported data do not show dose-response relationship, and/or the effects are seen only at relatively very high doses that can only be considered far beyond the human exposure range.

The study from Nakamura et al. (2015) that determined the effects of maternal and lactational exposure to BP-3 on development and reproductive organs of offspring of time-mated female Harlan Sprague-Dawley rats has been selected by the SCCS as the key study to be used for the calculation of the MoS. Due to the effects on the spermatocytes, which may be due to an ED effects of BP-3, the SCCS considered 3000 ppm as a LOAEL and 1000 ppm as the NOAEL (67.9 mg /kg bw/day)

Safety evaluation (including calculation of the MoS)

The SCCS has a dermal absorption of 9.9% [mean (3.1%) + 2 SD (2*3.4%)] for the use of BP-3 as a UV filter at 6% for the calculation of the systemic exposure dose (SED) and the margin of safety (MoS) for sunscreen products. For other cosmetic products, the SCCS has used a dermal absorption of 8% [mean (4%) + 2 SD (2*2%)].

For the calculation of the margin of safety (MoS), the SCCS has used a NOAEL of 67.9 mg/kg bw/d from the Nakamura et al. (2015) study.

The SCCS has estimated the margin of safety (MoS) as **38** for the use of BP-3 at a concentration of 6% as a UV-filter in sunscreens for the whole body application (in the form of cream/lotion or pump spray, and 36 for sunscreen propellant spray), and 447 for the face, 317 for the hand and 1257 for lipstick application. The MoS has been estimated at **585** for the use of BP-3 at 0.5% to protect cosmetic formulations against sunlight.

The SCCS assessment did not cover the safety of BP-3 for the environment.

4. CONCLUSION

1. In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Benzophenone-3, does the SCCS consider Benzophenone-3 safe when used as a UV-filter in cosmetic products up to a maximum concentration of 6% and up to 0.5% in cosmetic products to protect product formulation?

On the basis of safety assessment, and considering the concerns related to potential endocrine disrupting properties of benzophenone-3 (BP-3), the SCCS has concluded that:

f. The use of BP-3 as a UV-filter up to a maximum concentration of 6% in sunscreen products, either in the form of body cream, sunscreen propellant spray or pump spray, is not safe for the consumer.
g. The use of BP-3 as a UV-filter up to a maximum concentration of 6% in face cream, hand cream, and lipsticks is safe for the consumer.
h. The use of BP-3 up to 0.5% in cosmetic products to protect the cosmetic formulation is safe for the consumer.

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Benzophenone-3 as a UV-filter in cosmetic products?*

In the SCCS's opinion, the use of BP-3 as a UV filter in the following sunscreen products is safe for the consumer up to a maximum concentration of:

a. 2.2% in body creams, in propellant sprays and in pump sprays, provided that there is no additional use of BP-3 at 0.5% in the same formulation for protecting the cosmetic formulation.
b. Where BP-3 is also used at 0.5% in the same formulation, the levels of BP-3 used as UV filter should not exceed 1.7% in body creams, in propellant sprays and in pump sprays.

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

It needs to be noted that the SCCS has regarded the currently available evidence for endocrine disrupting properties of BP-3 as inconclusive, and at best equivocal. This applies to all of the available data derived from *in silico* modelling, *in vitro* tests and *in vivo* studies, either considered individually or taken together. The SCCS considers that, whilst there are indications from some studies to suggest that BP-3 may have endocrine effects, the overall evidence is not conclusive enough at present for the SCCS to ascertain whether or not BP-3 is an ED substance, and this warrants further investigations.

The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of BP-3 for the environment.

5. MINORITY OPINION

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7. GLOSSARY OF TERMS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181

8. LIST OF ABBREVIATIONS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181

Annex - 1: Summary of the available in vivo studies on reproduction function and ED effects of BP-3

Number and reference	Species Strain Model	Routes	Dose, Exposure period	Group size	Outcomes reported				NOAEL/ LOAEL **derived by** the authors	SCCS Comments
					General toxicity	Reprotoxicity	Teratogenicity	ED related effects		
- 1- Prenatal developmental tox study Study period: 2004. Ref: 5, 33 in submission dossier OECD 414 GLP	Rat Wistar CrI: WI (Han)	Oral Gavage in corn oil	0, 40, 200 and 1000 mg/kg bw/day From GD5 to GD 19 observations at GD 20	25	No adverse effects in the dams	no significant difference in the number of implantation sites/litter, mean resorptions/litter, % litters with resorptions, number and weights of live fetuses, between the control and treated groups	No teratogenic effects Minor disturbance ad delay of ossification at 1000 mg/kg bw/day		NOAEL mat. = 200 mg/kg bw/d NOAEL dev. = 200 mg/kg bw/d	This study has been used in the previous SCCS opinion as the key study for calculation of the MOS.
- 2 - Nakamura et al. 2015 Birth Defects Res 104: 35-51 Study period:	Rat Sprague-Dawley	Food	, 0, 1000, 3000, 10 000, 25 000, 50 000 ppm, *i.e.* around 67.9, 207.1, 670.8, 1798.3,	7-8	↓ body weights of dams at 50 000 ppm at GD 10, 15 and 20; and at 25 000 ppm at GD15 ↓ organ (liver, kidney) weights (absolute	no significant difference in the number of implantation sites/litter, mean resorptions/litter, % litters with resorptions, number and weights of live	One foetus with hydrocephaly at 50 000 ppm but no other malformations observed	In male offspring at PND23: ↓ AGD in male at 50 000 ppm ↓ spermatocytes per seminiferous tubules at 3000 ppm and higher	NOAEL : 3 000 ppm (670 mg/kg bw/day LOAEL : 10 000 ppm (1798 mg/kg/d)	It would have been useful to have measured foetal testosterone as it is known that a decrease during specific windows of sensitivity in male foetuses mat impaired the development of reproductive

			and 3448.2 mg/kg/day . from GD6 to PND 23 Observation on PND1 and PND 23		and relative) in female and male offspring and blood biochemical factors in the high dose (≥10,000 ppm) groups	fetuses, or sex ratios between the control and treated groups; ↑ ovaries and uterine weights (absolute and relative) at 1000 ppm but not at higher doses		doses (dose related) ↑ apoptic cells in seminiferous tubules at 10 000 ppm and above (not dose related) Limited ↓ testo level at 3000 and 25 000 ppm In female offspring at PND23 Delayed follicular development at 10 000 and 50 000 ppm		system (↓ AGD, ↓ spermatocytes production and/or quality…) Due to the effects on the ↓ of spermatocyte which may be due to an ED effects of BP-3 SCCS considered 3000 ppm as a LOAEL and therefore the NOAEL should be 1000 ppm (68 mg /kg bw/day)
- 3 - Submission Ref 11 Study period: 2012 (final report in 2015) Equivalent to OECD 414 GLP	Rat Sprague-Dawley	Oral Food	0, 3000, 10 000 and 30 000, ppm, *i.e.* around 242, 725 and 3689 mg/kg/ bw/day From GD 5 to GD 15 Observation at GD 15	25	.↓ body weights at 10 000 and 30 000 ppm and ↑ food consumption at 30 000 ppm modifications of blood biochemical factors at 30 000 ppm	No effects on foetuses, litters	No teratogenic effects observed excepted increased skeletal variations not considered treatment related		NOAEL mat. = 242 mg/kg bw/d NOAEL dev. = 3689 mg/kg bw/d	SCCS agrees with the authors conclusion.

- 4 - NTP modified 1-gen study equivalent to perinatal developmental tox study Study period : 2010-2012 (final report in 2020) Ref 21 in submission dossier GLP	Rat Sprague-Dawley	Oral Food	0,1000,3000and 10 000, ppm, *i.e.* ± 70, 206 and 660 mg/kg/ bw/day during gestation and 157, 478 and 1609 mg/kg bw/day during lactation . from GD6 until PND 21 for F0 and then during 105 weeks for F1 observation at PND1, 4, 7 , 14 and 21	35-42 females for F0 and 50-60 for F1 (m and f)	At 10 000 ppm: ↓ body weights gain in F0 at GD6-9 and lactation day 4-21 At 3000 ppm: ↓ body weights gain in F0 at GD 18 -21 ↓ body weights of F1 m and f at 10 000 ppm over the course of the study and at 3000 ppm in f at week 77	No effects on number of pups, litter size, sex distribution, In female F1: ↑ stromal polyp and endometrium hyperplasia ppm in the uterus at 3000 In male F1: ↑ fibirnoid necrosis of the arterioles at 10 000 ppm at the end of the 2 years	No teratogenic effects except at the highest dose: skeletal aberrations in the presence of maternal toxicity	↑ incidence of C-cell adenoma in the thyroid gland at 3000 ppm in f at the end of the 2 years	NOAEL mat. = 200 mg/kg bw/d NOAEL dev. = 200 mg/kg bw/d	The SCCS agrees with the derived NOAEL values.
- 5 - Ref 21 in submission dossier	Mouse B6C3F1/N	Oral Food	0, 1000, 3000, and 10000, ppm, *i.e.* around	50 m and 50 f per dose	↓ body weights of F1 m and f at 10 000 ppm over the					

NTP modified 1-gen study equivalent to perinatal developmental tox study Study period : 2010-2012 (final report in 2020) GLP			113, 339 and 1207 mg/kg/ bw/day for males and 109, 320 and 1278 mg/kg bw/day for females . from GD6 during 39 days for F0 and then during 105 weeks for F1 Sacrifice at 14 weeks for 10 F1 rats (m and f) at 0 and 10 000 ppm		course of the study ↑ incidence of pigment in bone marrow at 10 000 ppm in m and f ↑ incidence of pigment in spleen at 10 000 ppm in m and f ↑ incidence of syncytial alteration in the liver of males at all doses ↑ incidence of renal alterations at 3000 and 10 000 ppm in males ↑ incidence of osseous metaplasia in females at 10 000 ppm					
- 6 -	Rats	Oral Food	0, 3125,625,1					in males:	NOAEL = 411 mg/kg/ day	

Ref 5, 7, 33 in submission dossier Sub-chronic toxicity study OECD 408 GLP			2500, 25000 and 50000 ppm , ie ± 204, 411, 828, 1702 and 3458 mg/kg bw/day 13 weeks					↓ in epididymal sperm density, count and decrease in absolute cauda, epididymal and testis weights, reduced sperm motility at 3458 mg/kg bw/day In females: ↑ estrous cycle length in female rats at 3458 mg/kg bw/day	based on effects in the kidney	
- 7 - Ref 7, 16, 33 in submission dossier Sub-chronic toxicity study OECD 408 Non GLP	Rats	Oral Food	0, 0, 0.02, 0.1, 0.5 and 1% in the diet ie ± 0, 20, 100, 500 and 1000 mg/kg bw/day 13 weeks					In males and females: ↓ in absolute weights of adrenal gland, pituitary gland and gonads at 1000 mg/kg bw/day ↑ in relative thyroid and pituitary and adrenal weights at 500 and 1000 mg/kg bw/day	NOAEL = 100 mg/kg bw/day	

- 8 - Ref 5, 7, 33 in submission dossier Sub-chronic toxicity study OECD 408 GLP	Mice	Oral Food	0, 3125,6250, 12500, 25000 and 50000 ppm , ie ± 0, 554, 1246, 2860, 6780 and 16238 mg/kg bw/day 13 weeks			No effects on reproductive parameters at 6780 mg/kg bw/day or below		Males: ↓ in sperm density and ↑ in abnormal sperm morphology at 16238 mg/kg bw/day Females: ↑ in estrous cycles length at 16238 mg/kg bw/day	NOAEL = 1246 mg/kg bw /day based on systemic effects	
-9- Ref 21 Sub-chronic toxicity study GLP	Rats		0 and 10000 ppm ie ± 585/632 mg/kg bw/day in males and females					Estrogen-responsive genes showed no change in response to BP-3 in transcriptome analysis		
-10- Ref 5, 7, 33 in submission dossier Sub-chronic toxicity study ± OECD 411 GLP	Rats	Dermal	0, 12.5, 25, 50, 100 and 200 mg/kg bw/day 13 weeks			No effects on reproductive parameters			NOAEL = 200 mg/kg bw/day	
-11-	Mice	Dermal	0, 22.8, 45.5, 91,					↓ in epididymal sperm density	NOAEL = 364 mg/kg bw/day	In view of the effects on sperm

Ref 5, 7, 33 in submission dossier Sub-chronic toxicity study ± OECD 411 GLP			182, 364 mg/kg bw/day					at all doses which was not considered treatment related by the authors		density and in the absence of the original report, it is not possible for the SCCS to interpret this study.
- 12 - Laplante et al. 2018	2-part study Acute and chronic, in mice (6 to 8-week old female Balb/C ovariecto mised)	oral	Acute study: 4 days (3000 µg/kg/d, n=6). 17β-estradiol (250 µg/kg/d, n=4) used as a positive control. Negative controls (vehicle and nulliparous female mice). Chronic study: (30, 212 and 3000 µg/kg/d) from		At 5 weeks post-weaning mice killed and mammary glands collected.	Acute study: No effect on uterus weight at 3000 µg/kg/d, significant but modest ↑ in the expression of ERα. Chronic study: No effects on the number of pups, only modest non-significant change in length of gestation. No effect on the ductal density at 3000 and 30 µg/kg/d, ↓ ductal densities of mammary glands in 212		The authors concluded that BP-3 may alter mammary gland morphology and histomorpholog y.		The SCCS has noted that the results presented in the study are often in contradiction with the authors' conclusions in regard to dose response, non-monotonic dose response, estrogen and progesterone time of response etc, and do not demonstrate a clear correlation between the treatment and the measured parameters. Therefore, whilst the study may indicate potential endocrine activity of BP-3, they do not provide any clear

			Gestational Day (GD) until Lactational Day (LD21).			µg/kg/d females. More epithelium in mammary glands at 212 mg/kg /d or 3000 mg/kg/d, no difference in 30 mg/kg/d group. The expression of Ki67 ↑ in the BP-3 treated females. No difference in the expression of Esr-1 in mammary epithelium, PR mRNA expression, or ER beta-mediated gene expression.				scientific evidence that may be used in safety assessment of BP-3.
- 13-Matouskova et al. 2019	Male and female 6-8-week old BALB/c mice exposed during gestation and	Oral	4 groups (0, 30, 212, and 3000 µg/kg/day, orally dosed daily by from the pregnancy			No effect of BP-3 in males on bw, ↓ AGI in 30 µg and 212 µg male groups at PND21 and in puberty, but no differences in adult males, at		The authors concluded that low doses of BP-3 altered AGI in both males and females, decreased the size and complexity of		Same comments as for Laplante et al. (2018) study.

	perinatal periods. The study calculated anogenital index (AGI) and mammary glands from females collected, and measured the expression of Erα, Ki67 and progesterone receptors.		day-zero until the day before weaning (on lactational day-21), and offspring evaluated at 3 life stages - prior to puberty, at puberty, and in adulthood.			PND 21 ↓ size of the mammary gland epithelium in males but no difference at puberty. In females, no effect on bw at PND21 but 212 and 3000 µg/kg/day females were heavier at puberty. No effect on AGI in females on PND21, a ↓ in AGI 212 µg/kg/day females at puberty but no effect in adulthood. Modest↑ in ductal areas of mammary glands in females at PND21. At puberty, the females exposed to 30		the mammary ductal epithelium in males, and increased the appearance of alveolar buds in adult females. BP-3 altered the expression of hormone receptors in female mammary gland in an age- and dose-specific manner, and disrupted proliferation in the mammary epithelium.		

						and 3000 µg BP-3/kg/ day had a modest increase in the size of the ductal tree and a significant increase in the extent of growth into the mammary fat pad. A ↓ in cells positive for ERα in mammary gland of BP-3 exposed females only at 30 or 212 µg/kg/day, no differences in expression of progesterone receptor at PND21 or adulthood. A ↓ in 212 µg BP-3/kg/ day females at puberty, expression of Ki67 ↓ only in females from				

						30 µg/kg/day group.				
- 14-Santamaria et al., 2020	Female mice (C57BL/6J), and male mice (BALB/c)	Dermal	Groups of 19 mice treated at 50 mg/kg bw/day (or control) from GD0 to GD6; BP-3 measured in blood and amniotic fluid; weights of pups measured from PND1 to PND19 every 3 days after 2 pregnancies.			After the first pregnancy: No differences implantation areas at GD5, GD8, GD10 and GD12; no effects on the number of implantations, abortions, and abortion rates; fetal weight ↓ at GD14; 16.13% of foetuses affected by intrauterine growth restriction; altered pattern in maternal arteria uterine at GD10; male offspring from BP-3 exposed mice lighter at PND4 and from PND10 onwards; females		The authors concluded that The results suggest that dermal exposure to BP-3 at a low internal dose during early pregnancy could impair fetal development, resulting in growth-restricted fetuses. The intrauterine growth restriction (IUGR) phenotype of the fetuses was associated with transient changes in hemodynamic parameters at maternal uterine artery, reduced weight of male offspring and		The SCCS considers that whilst this study indicates a potential reproductive effects of BP-in mice, the observations need to be consolidated through further research, and for the time being it does not provide any clear scientific evidence that can be used in safety assessment of BP-3.

						offspring of BP-3 exposed mice showed decreased weight at PND1, PND10 and PND13 (recovered from PND16 onwards). The sex ratio of the offspring modified with more females than males. After the second pregnancy: No effect on implantations and abortion numbers; placenta weights ↓ at GD14 but not at GD10; no difference in fetal weight or fetus/placenta index; no modification of offspring weight curves; modified sex ratio with more		persistence of significant levels of BP-3 in amniotic fluid until GD14. A very interesting observation done in our studies was an alteration of the sex ratio of the progeny, with females predominating over males when analyzing both pregnancies.		

						females than males in the offspring of BP-3 treated mice.				

Annex -2: Calculation of the Systemic Exposure Dose (SED)

From dermal exposure:

Description	**Parameter**	**Sunscreen**	**Face cream**	**Hand cream**
UV filter uses (6%)				
Amount Applied	Q	18000 mg	1540	2160
Concentration	C	6%	6	6
Absorption through the skin	DA_a	9.9%	9.9	9.9
Dermal absorption per treatment (mg/kg bw/day)	$E*C*0.01*DA_a*0.01/60$	1.782	0.15246	0.21384
			Total SED from uses as UV filter:	**2.148**
Non-UV filter uses (0.5%)				
Description	**Parameter**	**all products**		
Amount Applied	Q	17400 mg		
Concentration	C	0.5%		
Absorption through the skin	DA_a	8%		
Dermal absorption per treatment (mg/kg bw/day)	$E*C*0.01*DA_a*0.01/60$	0.116		
			Total SED from uses as UV filter and non-UV uses:	**2.264**

From inhalation exposure:

Description	Parameter	sunscreen propellant spray	Pump spray	Unit
Amount by application*	A	15000	9000	mg/application
Fraction of BP-3 in non-propellant	C	0.06	0.06	fraction w/w
Proportion of non-propellant in formulation (60% for aerosol)	P	0.6	1	non-propellant in formulation
Airborne fraction	AF	1	0.2	fraction
Potential amount to be inhaled	**EA**	**540**	**108**	**mg**
Near-field, 1 m^{2}**	V_1	1000	1000	L
	BR	13	13	L/min
First step: 2 min in near-field	t_1	2	2	min
	IA_1	**14.04**	**2.808**	**mg**
Second step: 10 min in far field	V_2	10000	10000	L
	BR	13	13	l/min
Far-field, 10 m^2	t_2	10	10	min
	IA_2	**7.02**	**1.404**	**mg**
Substance availability (25% exhaled)	G	0.75	0.75	
Respirable fraction	RF	0.2	0.01	
Frequency of application	DA	2	2	
Default bodyweight	BW	60	60	kg
SED_{inhal}		**0.1053**	**0.001053**	**mg/kg bw/day**

*Adjusted for the proportion of propellant to achieve a final "on-body" amount of 9000 mg
** The SCCS considers that for exposure estimates of spray products the near field should be 1 m2.

From oral exposure:

Description	Parameter	Lip-stick	Unit
Relative daily exposure	$E_{product}$	0.9	mg/kg bw/d
Concentration of BP-3 in product	C	6	%
Potential amount available for oral exposure (retention factor)	F_{ret}	100	%
Adjusted for oral bioavailability		100	%
SED_{oral}		**0.054**	mg/kg bw/day