Scientific Committee on Consumer Safety

SCCS

OPINION on

Benzophenone - 1

(CAS No. 131-56-6, EC No. 205-029-4)

The SCCS adopted this document
during the plenary meeting on 25 October 2024

ACKNOWLEDGMENTS

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

For the Preliminary Opinion

SCCS members
Dr U. Bernauer
Dr L. Bodin
Prof. Q. Chaudhry (SCCS Chair and Rapporteur)
Prof. P.J. Coenraads (SCCS Vice-Chair, Chairperson of the WG)
Dr J. Ezendam
Dr E. Gaffet
Prof. C. L. Galli
Prof. E. Panteri
Prof. V. Rogiers (SCCS Vice-Chair)
Dr Ch. Rousselle
Dr M. Stepnik
Prof. T. Vanhaecke
Dr S. Wijnhoven

SCCS external experts
Dr E. Benfenati (Rapporteur)
Dr N. Cabaton
Prof. E. Corsini
Dr A. Koutsodimou
Dr M. Henriqueta D.L.G. Louro
Prof. W. Uter
Dr N. von Goetz

All Declarations of Working Group members are available on the following webpage:
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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-1, does the SCCS consider Benzophenone-1 safe when used as a light stabilizer in cosmetic products up to a maximum concentration of 2%?

Having considered the data provided, and the concerns relating to potential endocrine disrupting properties of Benzophenone-1, the SCCS cannot conclude on the safety of BP-1, because the information provided is insufficient to exclude genotoxicity.

The available evidence also shows that BP-1 is an endocrine-active substance due to clear demonstration of estrogenic activity and weak anti-androgenic activity both *in vitro* and *in vivo*, and potential activity against thyroid modality *in vitro*.

(2) Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Benzophenone-1 in cosmetic products?

/

(3) Does the SCCS have any further scientific concerns with regard to the use of Benzophenone-1 in cosmetic products?

The SCCS mandate does not address environmental aspects. Therefore, this assessment did not cover the safety of BP-1 for the environment.

Keywords: SCCS, scientific opinion, benzophenone – 1, Regulation 1223/2009, CAS No. 131-56-6, EC No. 205-029-4

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

SCCS

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

Scientific Committee members

Ulrike Bernauer, Laurent Bodin, Qasim Chaudhry, Pieter Jan Coenraads, Janine Ezendam, Eric Gaffet, Corrado Lodovico Galli, Eirini Panteri, Vera Rogiers, Christophe Rousselle, Maciej Stepnik, Tamara Vanhaecke, Susan Wijnhoven

Contact:
European Commission
Health and Food Safety
Directorate B: Public Health, Cancer and Health security
Unit B3: Health monitoring and cooperation, Health networks
L-2920 Luxembourg
SANTE-SCCS@ec.europa.eu

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background on substances with endocrine disrupting properties

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

Background on Benzophenone-1

Benzophenone-1 (CAS No. 131-56-6, EC No. 205-029-4) with the chemical name '2,4-dihydroxybenzophenone' is included in the European database for information on cosmetic substances and ingredients (CosIng) with the reported function of 'light stabilizer' and 'UV-absorber'. Benzophenone-1 is used in various rinse-off and leave-on cosmetic products since it is able to absorb and disperse ultraviolet (UV) radiation, protecting, therefore, the respective product formulation from the damaging effects of UV radiation. Currently, Benzophenone-1 is not regulated under the Cosmetic Regulation (EC) No. 1223/2009.

During the call for data, stakeholders submitted scientific evidence to demonstrate the safety of Benzophenone-1 as a light-stabilizer in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on Benzophenone-1 in view of the information provided.

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

Terms of reference

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Benzophenone-1, does the SCCS consider Benzophenone-1 safe when used as a light stabilizer in cosmetic products up to a maximum concentration of 2%?*

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Benzophenone-1 in cosmetic products?*

3. *Does the SCCS have any further scientific concerns with regard to the use of Benzophenone-1 in cosmetic products?*

3. OPINION

Preamble

This Opinion is based on the assessment of available scientific evidence regarding the safety of benzophenone-1 (CAS No. 131-56-6, EC No. 205-029-4) when used as a UV absorber and/or light-stabiliser in cosmetic products. As required by the European Commission's mandate, the available evidence has also been appraised for potential endocrine effects of benzophenone-1 (hereinafter referred to as BP-1).

It is notable from the submission that, due to gaps in the available data for BP-1, the Applicant proposed a case for data read-across from a close analogue BP-3. This approach was used by the Applicant for data gap filling for almost all toxicological endpoints – i.e. toxicokinetics, dermal absorption, acute dermal toxicity, repeated dose toxicity, skin sensitisation, reproductive/ developmental toxicity, mutagenicity/ genotoxicity (including photomutagenicity/ photoclastogenicity), carcinogenicity, and endocrine effects.

In this regard, the Applicant stated that the read-across has been carried out in accordance with the ECHA analogue justification guidance (2017) that requires commonality between the target and the source substances in terms of functional groups and structures; structural alerts or reactivity; physicochemical properties; and the breakdown products resulting from biological/degradation processes. It was argued that BP-1 and BP-3 share a high structural similarity (Dice index = 0.85), similarities in the key functional groups, (ketone and phenol); as well as similarities in the structural alerts identified by the profilers contained within the OECD (Q)SAR Toolbox. Also, that both substances belong to Cramer Class III, and that BP-1 is the main metabolite of BP-3.

In the view of the SCCS, the manner in which read-across has been presented does not fulfil the critical requirements for an unbiased and transparent read-across - i.e. no details have been provided on how analogues were searched, how many were identified, and on what basis all others except BP-3 were deselected from use in the read-across. The SCCS is therefore of the view that the proposed read-across from a single analogue (BP-3) can only be considered acceptable if it is not the only evidence for a given endpoint – i.e. there is more information from other line(s) of evidence to support the read-across outcome. This means that the SCCS has considered the data from read-across on an endpoint-to-endpoint basis to see whether or not it can be accepted as part of a collective weight of evidence for the given endpoint.

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Benzophenone-1

3.1.1.2 Chemical names

IUPAC name
(2,4-dihydroxyphenyl)-phenylmethanone

Other chemical names
2,4-Dihydroxybenzophenone
Benzoresorcinol
4-Benzoyl Resorcinol
Resbenzophenone

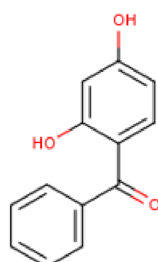
3.1.1.3 Trade names and abbreviations

Uvinol 400
Methanone
Syntase 100
Dastib 263
Advastab 48
BP-1
DHBP

3.1.1.4 CAS / EC number

CAS: 131-56-6
EC: 205-029-4

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{13}H_{10}O_3$

3.1.2 Physical form

Light-yellow, crystalline solid

3.1.3 Molecular weight

214.22 g/mol

3.1.4 Purity, composition and substance codes

The following is the only information provided by the Applicant:

Maximum moisture content-2%

3.1.5 Impurities / accompanying contaminants

Arsenic: 1 ppm; Lead: 18 ppm; Cadmium: 3 ppm; Magnesium: 1 ppm; Toluene: 900 ppm

(CIR, 2021)

SCCS comment
Only limited data on the heavy metal content of the test substance were provided. A full report on the impurities of the test substance in representative batches must be provided, including impurities from the synthesis route.

3.1.6 Solubility

Soluble in methanol, ethanol, ethyl acetate, methyl ethyl ketone, acetone, ether, and acetic acid; slightly soluble in benzene; insoluble in water

EpiSuite model estimated water solubility (at 25°C): 413.4 mg/L (CIR, 2021)
Water (at 22°C): 17.2 mg/L using UV/Vis (Ezzo, 2021; PC-Solubility Report)
Water (at 25°C): 235.6 mg/L (ECHA)

3.1.7 Partition coefficient (Log Pow)

2.964 at 25°C (ECHA, 2021a)
2.49 at 25°C (Ezzo, 2021; PC-LogP Report)

3.1.8 Additional physical and chemical specifications

Where relevant:
- organoleptic properties: odourless
- melting point: 144°C
- boiling point: 375°C at 101 325 Pa (ECHA), 194 °C at 1 mm Hg (PubCHem)
- flash point: 125°C
- vapour pressure: 0 Pa at 20°C (ECHA)
- density:
- relative density: 1.274 at 20°C
- viscosity: /
- pKa: 7.75 at 20°C (ECHA)
- pH:

- refractive index:
- UV/visible light absorption spectrum
- UV absorption λmax (nm): 290

ECHA: https://echa.europa.eu/el/registration-dossier/-/registered-dossier/12687/4/4
PubChem: https://pubchem.ncbi.nlm.nih.gov/compound/2_4-Dihydroxybenzophenone

3.1.9 Homogeneity and Stability

No data.

SCCS comment
Data on the stability of the test substance under the conditions of use must be provided.

SCCS comment on physicochemical properties
Data on purity, pH, and stability under use conditions should be provided.

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

According to the Applicant:

- No dermal absorption studies could be identified for BP-1.
- Based on its physicochemical parameters (i.e., molecular weight of 214.22 g/mol, water solubility of 17.2 mg/L and log KOW of 2.49), BP-1 is expected to have a low to moderate absorption potential via the dermal route (Kroes et al., 2007). For the purpose of safety assessment, a maximum flux value (Jmax) of 0.158 µg/cm2/h was calculated by multiplying the experimental water solubility (17.2 mg/L) with the predicted Kp value (0.0092 cm/h) from DERMWIN v2.02, (USEPA, 2021) indicating a maximum dermal absorption value of 40% (Kroes et al., 2007; Shen et al., 2014).
- In the experimental data with the analogue BP-3, a dermal absorption of 9.9% [mean (3.1%) + 2 SD (2*3.4%)] has been established (SCCS, 2021a).
- The Applicant decided to use a conservative approach based on the **default dermal penetration value of 50%** to estimate BP-1 exposure via its use in cosmetics, in line with the SCCS Notes of Guidance.

3.2.2 Oral absorption/bioavailability

According to the Applicant:

- No relevant *in vivo* or *in vitro* toxicokinetic studies on BP-1 could be identified in the literature. Therefore, the oral absorption or bioavailability of BP-1 was assessed based on physicochemical properties, structure activity information and toxicokinetic data available for BP-3.
- Based on its physicochemical parameters (i.e., molecular weight of 214.22 g/mol, water solubility of 17.2 mg/L and log KOW of 2.49), BP-1 is assessed to have a moderate oral absorption potential. The 'Lipinski's rule OASIS profiler'4 of the OECD (Q)SAR Toolbox v.4.4.1 predicts BP-1 to be 'bioavailable' (OECD, 2020).
- This PC- and QSAR-based assessment for BP-1 is further supported by toxicokinetic data available for the analogue BP-3. The following summary was reported in the recent SCCS opinion on BP-3:

"BP-3 was well absorbed following a single gavage administration of [14C]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 faeces (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)."

- Form the above information, BP-1 was considered to be well absorbed following oral exposure.
- In the absence of quantitative absorption data, the Applicant decided to adopt a conservative approach and use **a default oral absorption of 50%** as recommended in the SCCS NoG for the risk assessment/MoS calculations.

SCCS comments
In the absence of experimental data, much of the provided information on toxicokinetic aspects and dermal absorption has been drawn from physicochemical parameters and modelling predictions. Noting the absence of relevant data on toxicokinetics of BP-1, the SCCS has accepted the Applicant's proposed use of 50% default values for dermal absorption and 50% for oral absorption of BP-1 for use in safety assessment.

3.2.2 Other studies on toxicokinetics

According to the information provided by the Applicant:

Metabolism

- The OECD QSAR Toolbox v.4.4.1 predicts BP-1 to undergo aromatic hydroxylation and ketone reduction as first metabolic reactions. BP-3 is predicted to undergo O-dealkylation, giving rise to BP-1 (see Annex 1), as well as hydroxylation and ketone reduction. This is supported by the available literature data for BP-3, which indicated BP-1 as one of its major metabolites.
- Under *in vitro* conditions, BP-1 was reported to be formed when BP-3 (0.1 µmol) was incubated for 15 min with liver microsomes from untreated Sprague-Dawley rats in the presence of NADPH. No quantitative information was provided. The identification of BP-1 as a metabolite of BP-3 is further supported by other *in vitro* investigations in rat and human liver microsomes.

(Watanabe *et al.*, 2015; Kamikyouden *et al.*, 2013)

- Under *in vivo* conditions, BP-3 was also shown to give rise to BP-1 upon O-dealkylation in rats and humans via oral and dermal routes BP-1 was reported to be further metabolised to 2,3,4-trihydroxybenzophenone via aromatic hydroxylation and conjugation reactions with glucuronic acid and/or sulphate.

(El Dareer *et al.*, 1986; Jeon *et al.*, 2008; Kadry *et al.*, 1995; Okereke *et al.*, 1993; Okereke *et al.*, 1994)

- The conjugation potential of BP-3 metabolites was demonstrated in a recent NTP study in rats where plasma concentration determinations of free (unconjugated analytes) and/or total (free and all conjugated forms) metabolites of BP-3, including BP-1, indicated higher concentrations of the total forms (100- to 300-fold) compared to the free forms.

(SCCS, 2021)

- Furthermore, biomonitoring studies with BP-3 also indicated that BP-1 is a major metabolite, which was confirmed by the presence of BP-3 metabolites (including BP-1) in urine, suggesting demethylation as the major route of metabolism in humans.

(CIR, 2021)

Distribution
According to the Applicant:
Once in systemic circulation, given its low molecular weight and moderate log Kow, BP-1 is expected to be distributed to different organs. This is supported by the toxicokinetics data available on BP-3. Tissue analysis at 6 hours after oral administration of BP-3 in male Sprague–Dawley rats showed that BP-1, the major metabolite, was present in most tissues including the liver, kidney, testes, intestine, spleen and skin.

(Okereke *et al.*, 1993)

Excretion
According to the Applicant:
Considering its extensive conjugation, BP-1 is expected to be primarily eliminated via urine. This is demonstrated by the toxicokinetics data available on BP-3 and by human biomonitoring studies.

(CIR, 2021; El Dareer et al., 1986; Kadry et al., 1995; Okereke et al., 1993)

SCCS comment on Toxicokinetics
BP-1 is predicted by the OECD QSAR Toolbox to undergo aromatic hydroxylation and ketone reduction. The information from model predictions, as well as *in vitro* and *in vivo* studies on the structural analogue BP-3, has indicated that the metabolism of BP-3 leads to formation of BP-1. Biomonitoring studies also indicated that BP-1 is a major metabolite of BP-3 in urine in humans. Further metabolism of BP-1 is reported to lead to production of 2,3,4-trihydroxybenzophenone, and conjugation with glucuronic acid and/or sulphate.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

According to the submission, BP-1 functions as light stabiliser in cosmetic products and intended to be used in a range of both leave-on and rinse-off products at varying concentrations.

(CIR, 2021)

SCCS comment
The information provided in the submission indicates that BP-1 is intended for use in products for Bathing and showering (bubble bath, bath preparations, bath soaps and detergents); Fragrances (cologne and toilet waters, other fragrance preparations); Hair care (tonics, dressings, other hair aids); Nail care (nail creams and lotions, nail polish and enamel); Skin care (cleansing creams, other skin care preparations, US drug products-acne face wash).

Estimates of consumer exposure from the use of BP-1 containing cosmetic products have been provided by the Applicant under calculation of systemic exposure dose (SED) in section 3.5.1.

3.3.2 Calculation of SED/LED

TOXICOLOGICAL EVALUATION

According to the Applicant:

An exhaustive literature search for toxicological information on BP-1 was carried out. The search using 'chemical name/structure' and specific toxicology-relevant keywords was conducted in databases in ChemEXPERT™, TOXNET, PubMed Toxicology, and Google Scholar. ChemEXPERT™ is a commercial expert database which covers the toxicological data inventories of key global regulatory databases in the European Union, United States, Canada, Australia and Asia. Priority was given to regulatory reviews conducted in recent years. The literature search identified data for BP-1 on the following endpoints:

- Acute oral toxicity
- Skin and eye irritation
- Skin sensitisation (HRIPT)
- Subchronic toxicity
- Ames test
- Phototoxicity/photoallergy

Toxicological data gaps relative to the SCCS NoG were identified for the toxicokinetics, dermal absorption, acute dermal toxicity, genotoxicity in mammalian cells, carcinogenicity, and developmental and reproductive toxicity endpoints.

These endpoints were addressed by means of **read across to data from analogues**, which were identified using the ECHA recommended tools (such as OECD (Q)SAR Toolbox (OECD, 2020), US EPA AIM model (US EPA, 2021) and the process described by (Wu et al., 2010) and (Blackburn and Stuard, 2014). Identified analogues with relevant toxicological data were evaluated for their suitability in accordance with the analogue justification guidance which exists under the European Chemicals Agency read-across assessment framework (RAAF) (ECHA, 2017), based on the following criteria:

- Common functional groups and structure
- Common structural alerts or reactivity
- Common physico-chemical properties
- Likelihood of common breakdown products via biological/degradation processes

Among several candidates, one analogue with available toxicological data, assessed as 'suitable with interpretation' according to (Wu et al., 2010) was identified: Benzophenone-3 (BP-3; CAS No. 131-57-7). In particular, as BP-1 is a major metabolite of BP-3, external human exposure to BP-3 results in systemic exposure to BP-1 ([Applicant's] Section 3.3.1).

With respect to the four criteria, this analogue contains structural and functional features similar to those of BP-1, with slight differences which were further evaluated with regard to their relevance to the hazard assessment of BP-1 (see also [Applicant's] Annex I and Section 3.3.1 for details):

- High Dice index (0.85) indicative of high structural similarity.
- Similar key functional groups, such as ketone and phenol groups; BP-3 contains in addition alkoxy and ether functional groups.
- Similar structural alerts: (Q)SAR analysis conducted using the OECD (Q)SAR Toolbox v.4.4.1 (OECD, 2020) revealed that BP-3 is classified as a Cramer Class III substance (high toxicity), as is BP-1. BP-3. BP-1 presents the same alerts, identified by the 'Repeated dose', '*In vivo* mutagenicity (Micronucleus) alerts by ISS', 'Oncologic Primary

Classification', 'Protein binding by Oasis', 'Protein binding alerts for skin sensitisation according to GHS', 'Protein binding alerts for skin sensitisation by OASIS', 'Estrogen receptor binding' and 'Skin irritation' profilers.

- Comparable physicochemical properties.
- Similar metabolic pathway, as predicted by OECD QSAR Toolbox v.4.4.1. BP-3 it is predicted to undergo O-dealkylation, giving rise to BP-1, and aromatic hydroxylation and reduction of the ketone group similar to BP-1. This is supported with data on the metabolism of BP-3 ([Applicant's] Section 3.3.1).

SCCS comment

As indicated in Preamble, the SCCS is of the view that the proposed read-across between BP-3 and BP-1 does not fulfil the critical requirements for an unbiased and transparent read-across - i.e. no details have been provided on how analogues were searched, how many were identified, and on what basis they were deselected or selected for use in the read-across. Therefore, the SCCS has considered that the proposed read-across from a single analogue (BP-3) can only be considered acceptable if it is not the only evidence for a given endpoint – i.e. there is further information from other line(s) of evidence to support the read-across outcome. This means that the SCCS will only rely on the use of data read-across from BP-3 to BP-1 for a given endpoint where it forms part of a weight of evidence.

3.4.1. Irritation and corrosivity

3.4.1.1 Skin irritation

Skin irritation in Rabbits

Guideline:	FHSLA procedure
Species/strain:	Rabbits / Albino
Group size:	6 (sex not specified)
Test substance:	BP-1
Vehicle:	Petrolatum and dimethyl phthalate
Batch:	Not specified
Purity:	Not specified
Dose applied:	0.5 mL
Concentration:	4, 8 and 16%
Route:	Dermal
Type of coverage:	Occlusive
Area of exposure:	Not specified
Duration of exposure:	24 hours
Observation:	24 and 48 hours
GLP:	Not specified
Study period:	1967

The dermal irritation and corrosion potential of BP-1 was investigated according to the procedure outlined by the Federal Hazardous Substances Labelling Act (FHSLA) in Albino rabbits. A single dose of 0.5 mL test substance was applied via a patch at concentrations of 4, 8 and 16% in petrolatum and dimethyl phthalate (DMP) occlusively for 24 hours. The application sites were examined for signs of erythema and oedema immediately and 24 hours after patch removal and scoring was done according to the Draize scale.

Results

Except for slight irritation with the test substance at 16% in DMP, no dermal reactions were observed. The Primary Dermal Irritation Index (PDII) was calculated to be 0.25. No other skin reactions were recorded in any animal at the application sites.

Conclusion
Under the conditions of the study, BP-1 was non-to minimally irritating to rabbit skin at a concentration up to 16%.

(CIR, 1983)

SCCS comments
The provided evidence includes a historic study in rabbits and two studies on human volunteers (details in section 3.4.9). It is notable that the level of BP-1 tested (1% and 0.5%) in the human studies is lower than the intended level of use in cosmetic products (2%). However, considering the collective evidence from the studies, the SCCS agrees that BP-1 is not likely to be a skin irritant at the proposed use levels in cosmetic products.

3.4.1.2 Mucous membrane irritation / eye irritation

Guideline: Not specified
Species/strain: Rabbits/ Albino
Group size: 6 (sex not specified)
Test substance: BP-1
Vehicle: Unchanged (no vehicle)
Batch: Not specified
Purity: Not specified
Dose applied: 100 mg
Concentration: 100% Duration of exposure: No eye wash
Observation: 7 days
GLP: Not specified
Study period: 1964

The eye irritation potential of BP-1 was investigated in in Albino rabbits. 100 mg undiluted test substance was placed into the conjunctival sac of the eye of each of 6 rabbits. The untreated eye served as control. The effects on the cornea, iris and conjunctivae were observed daily for 7 days after exposure to the test substance and scored according to the Draize scale.

Results
The average Draize score for irritation was calculated to be 20, 7 and 0 on Day 1, 2 and 3 respectively.

Conclusion
Under the conditions of the study, the BP-1 was mildly irritating to rabbit eyes.

(CIR, 1983)

Guideline: FHSLA procedure
Species/strain: Rabbits/ albino
Group size: 6 (sex not specified)
Test substance: BP-1
Vehicle: Petrolatum and dimethyl phthalate
Batch: Not specified
Purity: Not specified
Dose applied: 0.1 mL
Concentration: 4, 8 and 16%
Duration of exposure: No eye wash

Observation: 7 days
GLP: No
Study period: 1967

The eye irritation potential of BP-1 was investigated according to a protocol outlined by the Federal Hazardous Substances Labelling Act (FHSLA) in Albino rabbits. 0.1 mL of 4, 8 and 16% of test substance in DMP or petrolatum was placed into the conjunctival sac of the eye of each rabbit. The untreated eye served as control. The effects were observed daily for 7 days after exposure to the test substance and scored according to the Draize scale.

Results
No signs of eye irritation were noted during the study period in any animal with the test substance. The overall Draize scores was 0.

Conclusion
Under the conditions of the study, the BP-1 was not irritating to rabbit eyes.

(CIR, 1983)

SCCS comments
The SCCS has noted that the evidence from the two provided studies suggests that BP-1 is not likely to be an eye irritant at the proposed use levels in cosmetic products.

3.4.2 Skin sensitisation

There is no study available in respect to the skin sensitising potential of BP-1 in experimental animals. The skin sensitisation endpoint has therefore been assessed on the basis of human repeat insult patch test (HRIPT) data with Benzopehnone-1 as well as skin sensitisation data available for the analogue BP-3 in experimental animals (guinea pig, mouse) and humans. Studies on human volunteers have been detailed in section 3.4.9 (Human data).

3.4.3 Acute toxicity

3.4.3.1 Acute oral toxicity

Acute oral toxicity

Guideline: Not specified
Species/strain: Rats (strain not specified)
Group size: Not specified
Test substance: BP-1 (Ergostab 2H)
Vehicle: Olive oil
Batch: Not specified
Purity: Not specified
Doses: Not specified
GLP: No
Study period: 1968

BP-1 was investigated for acute oral toxicity in rats (strain not specified). Rats (sex not reported) were administered the test substance (doses not specified) in olive oil. The animals were observed for test substance related mortality.

Results

No mortalities were observed during the study. No further details on study results are available.

Conclusion

Under the conditions of this study, the LD50 of BP-1 was 8600 mg/kg bw (95% CL= 5930-12470) for rats.

(CIR, 1983; 2021)

Guideline:	Not specified
Species/strain:	Rats (strain not specified)
Group size:	50
Test substance:	BP-1
Vehicle:	Corn oil
Batch:	Not specified
Purity:	Not specified
Doses:	8-32 mL/kg (equivalent to approximately 8000-32000 mg/kg)
GLP:	Not specified
Study period:	1960

BP-1 was investigated for acute oral toxicity in rats. Fifty rats (sex not reported) were administered a single dose in the range of 8000-32000 mg/kg bw in corn oil. Following exposure, the animals were observed for 14 days. Necropsy with gross pathological examinations were performed after sacrificing the animals on Day 14.

Results

No details on mortality and other clinical signs were reported.

Conclusion

Under the conditions of this study, the LD50 of BP-1 was 24400 mg/kg bw.

(CIR, 1983)

3.4.3.2 Acute dermal toxicity

According to the Applicant:

No acute dermal studies could be identified for BP-1. The acute dermal endpoint has therefore been assessed on the basis of data available for the structural analogue BP-3.

Guideline:	Similar to OECD Guideline 402
Species/strain:	Rabbits / Albino
Group size:	5 animals / group (male)
Test substance:	BP-3
Vehicle:	No vehicle
Batch:	Not specified
Purity:	Not specified
Doses:	2000, 4000, 8000 and 16000 mg/kg bw
Route:	Dermal
Administration:	Topical
Observation period:	5 days
GLP:	No
Study period:	1953

The acute dermal toxicity of BP-3 was evaluated in a study similar to OECD Test Guideline 402. The substance was applied semi-occlusively at dose levels of 2000, 4000, 8000 and

16000 mg/kg bw to the shaved skin of male Albino rabbits (5/group) for an exposure period of 18-22 hours. The animals were observed for mortality, clinical signs of toxicity and gross pathological changes for 5 days.

Results
Mild skin irritations were noted in 2 animals of the low dose group (2000 mg/kg bw) and one animal from the dose group died at Day 4 of the observation period. Both observations, i.e., mild skin irritation and mortality, were not considered treatment related as these occurred only in the low dose group. No significant gross findings were noted following necropsy and autopsy. No mortality was observed up to the highest tested dose.

Conclusion
Under the conditions of the study, the acute dermal LD50 of BP-3 was greater than 16000 mg/kg bw for male rabbits.
(SCCP, 2006)
Based on an acute dermal toxicity study with BP-3, BP-1 is considered to be of low acute toxicity.

3.4.3.3 Acute inhalation toxicity

/

SCCS overall comment on Acute Toxicity
The limited available information from historic studies on acute toxicity in rats (oral) and rabbits (dermal) suggests that BP-1 is not likely to be acutely toxic.

3.4.4 Repeated dose toxicity

According to the Applicant:

Oral

Except for one subchronic toxicity study, no repeated dose toxicity studies could be identified for BP-1. Therefore, data available on BP-3 have been used for assessing this endpoint. The overview of the sub-acute and sub-chronic repeated dose studies with BP-3 are summarised in [Applicant's] Tables 2 and 3.

Oral Subchronic toxicity - BP-1

Guideline:	Not specified
Species/strain:	Rat/Albino
Group size:	5/sex/group (40 total)
Test substance:	BP-1 (Ergostab 2H)
Vehicle:	No vehicle
Batch:	Not specified
Purity:	Not specified
Route:	Oral
Administration:	Dietary
Doses:	0, 190, 600 and 1900 mg/kg bw/day
Duration:	90 days
GLP:	No
Study period:	Not specified

The oral subacute toxicity of BP-1 was investigated in Albino rats (5/sex/group). The animals were dosed daily via the diet at 0, 190, 600 and 1900 mg/kg bw/day for 90 days. During the treatment period, animals were observed for clinical signs, mortality, body weight and growth. At termination of treatment, all animals were sacrificed, macroscopically examined and organs were weighed and comprehensive histopathology was performed.

Results
Depressed growth rate, quantitative changes in erythrocyte and leucocyte numbers and lesions in liver and kidney were seen in the 600 and 1900 mg/kg bw/day dose group. No further details are available.

Conclusion
Under the conditions of the study, the NOAEL for BP-1 was established at 190 mg/kg bw/day in rats.

(Homrowski,1968)

Toxicity data of analogue BP-3
The repeated dose toxicity of BP-3 was evaluated in a range of guideline and non-guideline subacute and subchronic oral and dermal toxicity studies in rats and mice. The study designs, key results and NOAELs are summarised in Table 1.

Table 1:Overview of oral repeated dose toxicity studies with BP-3 as summarised in SCCP, 2006 and SCCS, 2021a

Study type, Species	Doses	Key findings	Reported NOAEL	Reference
14 days dietary toxicity study, F344/N rats (5/sex/group)	0, 3125, 6250, 12500, 25000 and 50000 ppm (corresponding to approximately 0, 303, 576, 1132, 2238 and 3868 mg/kg bw/day)	Increased liver weights with marked hepatocyte cytoplasmic vacuolization at 6250 ppm and higher. Increased kidney weights in males in all treatment groups except the low dose animals receiving 3125 ppm (i.e., 303 mg/kg bw/day).	NOAEL: 3125 ppm (i.e., 303 mg/kg bw/day)	(SCCP, 2006)
14 days dietary toxicity study, B6C3F1 mice (5/sex/group)	0, 3125, 6250, 12500, 25000 and 50000 ppm (corresponding to 0, 1021, 2041, 4430, 8648 and 20796 mg/kg bw/day)	Increased liver weights in males and females associated with the presence of cytoplasmic vacuolization of hepatocytes and increased kidney weights in males were recorded in all treatment groups except low dose. Decreased kidney weights in males at 25000 and 50000 ppm (i.e., 8648 and 20796 mg/kg bw/day).	NOAEL: 3125 ppm (i.e., 1021 mg/kg bw/day)	(SCCP, 2006)

13 weeks dietary toxicity study, (OECD Guideline 408, GLP), F344/N rats (10/ sex/ group)	0, 3125, 6250, 12500, 25000 and 50000 ppm (corresponding to approximately 204, 411, 828, 1702 and 3458 mg/kg bw/day)	Decrease in epididymal sperm density, count and decrease in absolute cauda, epididymal and testis weight as a consequence of reduced body weight and reduced sperm motility in male rats and significant increase in estrous cycle length in female rats in the highest dose group. No effects on the reproductive parameters at 1702 mg/kg bw/day or below.	NOAEL: 6250 ppm (i.e., 411 mg/kg/day; 393 for females and 429 mg/kg bw/day for males)	(SCCP, 2006)
13 weeks dietary toxicity study, (OECD Guideline 408, GLP), B6C3F1 mice (10/ sex/ group)	0, 3125, 6250, 12500, 25000 and 50000 ppm (corresponding to 0, 554, 1246, 2860, 6780 and 16238 mg/kg bw/day)	Decrease in sperm density, increase in abnormal sperm morphology in male mice and increase in estrous cycle length in female mice at high dose. No effects on reproductive parameters at 6780 mg/kg bw/day or below.	NOAEL: 6250 ppm (i.e., 1246 mg/kg bw/day)	(SCCP, 2006)
90-day dietary toxicity study (Similar to OECD Guideline 408, non-GLP), Wistar rats (12/ sex/ group)	0, 0.02, 0.1, 0.5 and 1% (corresponding approximatively to 0, 20, 100, 500 and 1000 mg/kg bw/day)	Decrease in absolute weights of the adrenal gland, pituitary glands and gonads in males and females at 1000 mg/kg bw/day. Increase in relative thyroid, pituitary and adrenal weights in both sexes at 500 and/or 1000 mg/kg bw/day. No changes in gonad or thyroid weights at 100 mg/kg bw/day or below.	NOAEL: 0.1% (i.e., 100 mg/kg bw/day)	(SCCP, 2006)

Following repeated oral administration of BP-3 in rats and mice, the most frequently encountered signs of systemic toxicity were retarded body weight gain, possibly triggered by reduced food consumption, paired with adverse effects in the kidney and liver. These effects were partly associated with changes in clinical chemistry including changes in liver enzymes, serum levels of proteins and haematological parameters. The most susceptible parameter was an increase in liver weight. The latter, however, was without any correlating histopathological findings and was therefore not considered to be an adverse effect but an adaptive response which is known to be reversible. The most suitable study to assess BP-3's repeated dose toxicity is a GLP compliant OECD Test Guideline 408 guideline subchronic feeding study in rats. In this study, BP-3 revealed decreases in epididymal sperm density, count and decrease in absolute cauda; epididymal and testis weight as a consequence of the reduced body weight and reduced sperm motility in male rats at 3458 mg/kg bw/day and above. Moreover, a

significant increase in estrous cycle length in female rats was observed in the highest dose group (3458 mg/kg bw/day). There were no effects on the reproductive parameters at 25000 ppm (i.e.,1702 mg/kg bw/day) or below.

Therefore, according to the SCCS 2006 opinion, the oral NOAEL was defined at 6250 ppm, corresponding to 411 mg/kg bw/day (i.e., 393 for females and 429 mg/kg bw/day for males) based on effects in the kidney.

Dermal

No repeated dose dermal toxicity studies could be identified for BP-1. The dermal endpoint was therefore assessed on the basis of data available for the analogue BP-3.

Toxicity data of analogue BP-3

A range of guideline and non-guideline subacute and subchronic dermal toxicity studies in rats and mice are available for the analogue BP-3. The study designs, key results and NOAELs are summarised in Table 2.

Table 2: Overview of dermal repeated dose toxicity studies with BP-3 as summarised in SCCP, 2006 and SCCS, 2021a

Study type, Species	Doses	Key results	Reported NOAEL	Reference
14 days dermal toxicity study, F344/N rats (5/ sex/ group)	0, 1.25, 2.5, 5, 10, or 20 mg/rat (equivalent to 0, 7, 13.6, 27.7, 54.9 and 110 mg/kg bw/day)	Small and variable increases in liver and kidney weights, primarily in the higher dose groups. No discernible histopathologic changes associated with the increases in liver or kidney weights.	NOAEL: 110 mg/kg bw/day	(SCCP, 2006)
14 days dermal toxicity study, B6C3F1 mice (5/ sex/ group)	0, 0.5, 1, 2, 4 and 8 mg/mice (equivalent to 0, 24.8, 48.4, 100, 196 and 388 mg/kg bw/day)	Statistically significant increase in liver weight at 196 and 388 mg/kg bw/day. No discernible histopathologic changes associated with increases in liver weights.	NOAEL: 388 mg/kg bw/day	(SCCP, 2006)
13 weeks dermal toxicity study (Similar to OECD Guideline 411, GLP), F344/N rats (10/ sex / group)	0, 12.5, 25, 50, 100, 200 mg/kg bw/day	No treatment related adverse effects in reproductive functions/parameters, i.e., sperm morphology/motility and vaginal cytology were observed up to the highest tested dermal dose of 200 mg/kg bw/day in rats.	NOAEL: 200 mg/kg bw/day	(SCCP, 2006)

13 weeks dermal toxicity study (Similar to OECD Guideline 411, GLP), B6C3F1 mice (10/ sex / group)	0, 22.8, 45.5, 91, 182, 364 mg/kg bw/day	Decrease in epididymal sperm density at all dose levels. The authors considered this effect as incidental in the absence of any other relevant findings in reproductive organs or related parameters.	NOAEL: 364 mg/kg bw/day	(SCCP, 2006)
4 weeks dermal toxicity study, rats	100 mg/kg bw/day	No effects on body weight, liver, kidney or testes weight, or histopathology. Exposure lowered glutathione-S-transferase levels in blood.	No NOAEL derived.	(Okereke *et al.*, 1994)

Inhalation

No inhalation studies could be identified for BP-1 or the analogue BP-3.

SCCS comments

The data provided on repeated-dose toxicity on BP-1 is limited to an old (1968) subchronic 90-day study in rats, for which details are not available to allow assessment of reliability of the reported results. However, this study indicated depressed growth rate, quantitative changes in erythrocyte and leucocyte numbers and lesions in liver and kidney in the high dose (600 and 1900 mg/kg bw/day) group. Other studies quoted by the Applicant relate to the analogue (BP-3) and not BP-1.

In view of the questionable quality and relevance of the provided data, the SCCS considers that the available evidence does not allow derivation of NOAEL, and therefore the SCCS does not agree with the proposed NOAEL of 190 mg/kg bw/day in rats from the study on BP-1.

3.4.5 Reproductive toxicity

3.4.5.1 Fertility and reproduction toxicity

According to the Applicant:

Except for a fertility study for which only limited information on methodologies and findings is available, no other reproductive nor developmental toxicity study could be identified for BP-1. Its reproductive and developmental toxicity was therefore assessed on the basis of data on the structural analogue BP-3.

In a fertility study, BP-1 was administered via oral, subcutaneous and intra-peritoneal administrations to female rats for 3 days. NOAEL of 100 mg/kg bw/day were reported for reproductive effects for the oral and intra-peritoneal routes and a NOAEL of 250 mg/kg bw/day via the subcutaneous administration. No further details are available.

(CIR, 2021; ECHA, 2021a)

1 3.4.5.2 Reproductive and developmental Toxicity
2
3
4
5 **Reproductive and developmental toxicity data of analogue BP-3**
6
7 According to the Applicant, several reproductive and developmental toxicity studies are
8 available on BP-3. The study designs, key results and NOAELs are summarised in Table 3.
9
10 Table 3: Overview of reproductive and developmental toxicity studies with BP-3 as
11 summarised in SCCP, 2006 and SCCS, 2021a

Study type, Species	Doses and exposure period	Key findings	Reported results/point of departure	Reference
Reproductive Toxicity (NTP Continuous Breeding Protocol), oral, mice	1850, 3950 and 9050 mg/kg bw/day/ continuous breeding	Reduced number of pups per litter and reduced dam weights at the two highest dose levels (3950 and 9050 mg/kg bw/day). However, no change in the average litters per pair. There were no effects on the reproductive parameters at 1850 mg/kg bw/day	NOAEL: 1850 mg/kg bw/day	(SCCP, 2006)
Modified One-Generation reproduction toxicity (NTP Protocol), dietary, rats	0, 3000, 10000 and 30000 ppm F0 females: From Gestation Day (GD) 6-21: 205, 697, and 2644 mg/kg bw/day; From Lactation Day (LD) 1-13: 484, 1591, and 5120 mg/kg bw/day; F1 rats (all cohorts; PND28-PND91): 267, 948, and 3003 (males) and 287, 983, and 9 3493 mg/kg bw/day	In the prenatal cohort, exposure to 30000 ppm was associated with significantly decreased mean numbers of corpora lutea and F2 implants. In the reproductive performance cohort, total F2 mean litter size on PND 0 was also significantly decreased compared to the control group. Exposure might have affected litter size, although the effect was small in magnitude. Collectively this was considered equivocal evidence of an adverse effect on reproductive performance. At 30000 ppm, significantly decreased F1 postweaning mean body	NOAEL: 3000 ppm (i.e., 215-577 mg/kg bw/day) based on based on a decrease in F2 litter size in both the prenatal and reproductive performance cohorts and postnatal growth retardation	(NTP, 2021)

	(females), respectively; adult F1 females: approximately 240, 825 and 2760 mg/kg bw/day (GD 0-21) and 426, 1621 and 5944 mg/kg/day (LD 1-13) F2 rats: PND 28 days	weights were not associated with concurrent lower feed consumption. The effects on body weights associated with exposure were considered some evidence of developmental toxicity. Diaphragmatic hernias were observed at a low incidence in both the F1 and F2 generations. F1 rats were associated with higher kidney weights, renal tubule epithelial regeneration, interstitial chronic active inflammation, renal tubule and pelvic concretions, renal tubule dilation, papillary necrosis, urothelial hyperplasia, and urothelial ulcers. F1 females also displayed renal tubule epithelial degeneration, pelvic dilation, chronic progressive nephropathy, and mineralization. At 30000 ppm, significantly decreased the absolute adrenal gland in female in the reproductive performance cohort. At 30000 ppm, F2 fetal findings of hydronephrosis of the kidney and enlarged liver were observed and exhibited dilation of the renal pelvis in F2 offspring. Under the conditions of this MOG study, there was some evidence of developmental toxicity in		

		rats based on the observed postnatal growth retardation.		
13 weeks, specific investigation study (male fertility), dermal, mice	0, 20, 100, 400 mg/kg bw/day	No treatment related adverse effects in reproductive functions/parameters, or the related parameters up to the highest tested dose of 400 mg/kg bw/day	NOAEL: 400 mg/kg bw/day	(SCCP, 2006)
Prenatal developmental toxicity (OECD Guideline 414; GLP), oral gavage, rats	0, 40, 200 and 1000 mg/kg bw/day / gavage / GD 5-19	No teratogenic effects observed after oral exposure to BP-3. Disturbance and delay of ossification at highest dose level attributed to maternal toxicity. No effects on the developmental or teratogenic parameters at 200 mg/kg bw/day or below.	NOAEL: 200 mg/kg bw/day	(SCCP, 2006)
Prenatal developmental toxicity (ICH segment-II; Similar to OECD Guideline 414; GLP), dietary, rats	3000, 10000 or 30000 ppm in diet (corresponding to 242, 725 and 3689 mg/kg bw/day) / GD 5-15	No adverse embryo/fetal developmental effects observed except for a statistically significant increase in total skeletal variations in the low dose group. However, this finding not considered treatment related as dose response not observed.	No other effects on the developmental or teratogenic parameters at 3689 mg/kg bw/day or below	(SCCS, 2021a)
Pre and post-natal study (NTP protocol), dietary, rats	0, 1000, 3000, 10000, 25000 and 50000 ppm in diet corresponding to approximately 0, 67.9, 207.1, 670.8, 1798.3 and 3448.2 mg/kg bw/day/ from GD 6 to Postnatal Day (PND) 23	Increased liver and kidney weights in dams observed. Clinical findings occurred primarily in the higher dose groups and often at all time points. No significant differences were observed in littering parameters. Male and female pups in the two highest dose groups displayed lower body weights. Decreased	Due to the effects on the spermatocytes, which may be an ED effect of BP-3, the SCCS considered 3000 ppm as a LOAEL and 1000 ppm as the NOAEL (67.9 mg /kg bw/day)	(SCCS, 2021a)

		normalized anogenital distance in male pups at postnatal day (PND) 23 and impaired spermatocyte development in testes of male offspring and delayed follicular development observed in the highest dose group (50000 ppm). NOAEL: 10000 ppm (670.8 mg/kg bw/day) for the reproductive system.		
NTP modified 1- gen study equivalent to Perinatal study (NTP protocol; GLP), dietary, rats	0, 1000, 3000, 10000 ppm in diet corresponding to approximately 0, 70, 206 and 660 mg/kg bw/day during gestation and 0, 157, 478 and 1609 mg/kg bw/day during LD 1-14)/ GD 6 to PND 21	Reduced body weight gain during GD 6–9 and lactation day 4–21 at 10000 ppm. Reduced pup body weight (approximately 10%) observed starting post-natal day 4 until weaning at the highest dose. No effects on the percentage of mated females producing pups, litter size, pup sex distribution, or numbers of male or female pups up to 3000 ppm (206-478 mg/kg bw/day)	Maternal NOAEL: 1000 ppm (i.e., 70 mg/kg bw/day) Developmental NOAE: 3000 ppm (i.e., 206-478 mg/kg bw/day)	(SCCS, 2021a)

The reproductive toxicity of BP-3 was assessed on the basis of two US National Toxicology Program (NTP) oral toxicity studies in rodents and one 13-week dermal male fertility study in mice via the dermal route. In the oral NTP Continuous Breeding Protocol in mice, a NOAEL of 1850 mg/kg bw/day was derived based on the reduced number of pups per litter and reduced dam weights at the two highest dose levels (3950 and 9050 mg/kg bw/day) (SCCP, 2006). Further, in the more recent NTP Modified One-Generation (MOG) study in rats, significantly decreased mean numbers of corpora lutea and F2 implants were noted at 30000 ppm (4460-6426 mg/kg bw/day). In the reproductive performance cohort, total F2 mean litter size on PND 0 was also significantly decreased compared to the control group. Although the study investigators did not establish study NOAELs, both reproductive and developmental NOAELs can be conservatively considered to be 3000 ppm (215-577 mg/kg bw/day) (NTP, 2021). In the 13-week dermal male fertility study in mice, a NOAEL of 400 mg/kg bw/day was established at the highest tested dose (SCCP, 2006). With regard to development toxicity, two pre-natal developmental as well as two pre-and post-natal toxicity studies in rats were available with BP-3. In the prenatal developmental toxicity studies via the oral route, BP-3 was not found to be teratogenic under the conditions of the test. Delayed ossification which

was noted at the highest dose occurred only in the presence of maternal toxicity in the pre-natal development toxicity study via the gavage route in rats. The NOAEL for maternal and developmental toxicity was determined to be 200 mg/kg bw/day (SCCP, 2006). The second ICH segment-II or pre-natal development dietary administration study with BP-3 confirmed the absence of treatment induced effects on the embryo or foetuses up to and including the highest tested dose of 3689 mg/kg bw/day (SCCS, 2021a).

Further, the findings from the pre- and post-natal studies in rats confirm the absence of test substance related teratogenic effects. In the pre- and postnatal study with BP-3, decreased normalized anogenital distance in male pups and impaired spermatocyte development in testes of male offspring and delayed follicular development was observed in the highest dose group (50000 ppm). The number of spermatocytes per seminiferous tubule was significantly reduced at doses of 3000 ppm and higher. Although, the study investigators derived a NOAEL at 10000 ppm (670.8 mg/kg bw/day) for the reproductive system, the SCCS considered the dose of 1000 ppm (67.9 mg/kg bw/day) as the NOAEL considering effects on spermatocytes at 3000 ppm (SCCS, 2021a). In pre and post-natal toxicity study, body weight changes in dams and pups were noted at higher doses (SCCS, 2021a). Although the study investigators did not establish study NOAELs, the maternal and developmental NOAELs can be assessed to be 1000 ppm (70 mg/kg bw/day) and 3000 ppm (206-478 mg/kg bw/day) respectively.

SCCS comments

The data provided on reproductive and developmental toxicity on BP-1 is limited to a fertility study in which BP-1 was administered via oral, subcutaneous and intra-peritoneal administrations to female rats for 3 days. Based on this study, a NOAEL of 100 mg/kg bw/day has been reported for reproductive effects for oral and intra-peritoneal routes, and 250 mg/kg bw/day via the subcutaneous administration. No further details are available for the study, and the provided information has been abstracted from CIR and EChA references. In the absence of full study report, the SCCS has not been able to assess reliability of the study. Other studies quoted by the Applicant relate to the structural analogue (BP-3) and not BP-1. In view of the unknown quality of the study on BP-1, and the questionable relevance of the information from BP-3 alone, the SCCS considers that the available evidence is not sufficient to support the proposed NOAELs for reproductive and developmental toxicity of BP-1.

3.4.6 Mutagenicity / genotoxicity

According to the Applicant:

The genotoxicity of BP-1 has been investigated in various bacterial *in vitro* mutagenicity assays. As the complete genotoxicity battery of tests data was not identified for BP-1, any endpoint data gaps were assessed on the basis of data available for the analogue BP-3.

3.4.6.1 Mutagenicity / genotoxicity *in vitro*

Bacterial Reverse Mutation Test (Ames)

Guideline:	OECD Guideline 471
Test system:	Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100
Replicates:	Not specified
Test substance:	Benzophenone -1
Vehicle:	DMSO
Batch:	Not specified
Purity:	Not specified
Test concentrations:	0.1- 500 µg/plate with and without S9-mix
Negative control:	Not specified

Positive control:	Not specified
GLP:	No
Study period:	1980

BP-1 was tested in an OECD Test Guideline 471 study to evaluate its mutagenic potential in Salmonella typhimurium tester strain TA 1535, TA 1537, TA 1538, TA 98 and TA 100, with and without metabolic activation (S9-mix). The concentrations of the test substance ranged from 0.1 to 500 µg/plate.

Results
The test substance did not show any mutagenic activity up to the highest concentration in the presence or absence of S9-mix.

Conclusion
Under the conditions of the study, BP-1 was not mutagenic in the bacterial reverse mutation test (Ames test), either in the presence or absence of S9-mix.

(CIR, 1983; ECHA, 2021b)

Guideline:	Not specified
Test system:	Salmonella typhimurium strains TA 98 and TA 100
Replicates:	Not specified
Test substance:	Benzophenone -1
Vehicle:	DMSO
Batch:	Not specified
Purity:	Not specified
Test concentrations:	600 µg/plate with and without S9-mix
Negative control:	Not specified
Positive control:	With S9-mix: Benzo[a]pyrene (BaP), and Without S9-mix: 2-(20-furyl)-3-(5-nitro-2-furyl) acrylamide (AF2)
GLP:	Not specified
Study period:	2006

The mutagenic potential of BP-1 was evaluated in an Ames test using S. typhimurium strains TA98 and TA100 with and without S9-mix. The concentrations of the test substance ranged up to 600 µg/plate.

Results
The test substance did not produce clear positive results with or without S9-mix.

Conclusion
Under the conditions of the study, BP-1 did not produce clear positive results with or without S9-mix and was classified as negative for mutagenicity.

(Nakajima et al., 2006)

Guideline:	Not specified
Test system:	Salmonella typhimurium strains TA 97, TA 98, TA 100, and TA 102
Replicates:	Triplicates
Test substance:	Benzophenone -1
Vehicle:	DMSO
Batch:	Not specified
Purity:	99%
Test concentrations:	0.05, 0.5, 5, 50 and 500 µg/plate with and without S9-mix

Negative control: Blank and DMSO control
Positive control: With S9-mix: 2-AF, Dantron Without S9-mix: NaN3, Dexon
GLP: No
Study period: 2018

BP-1 was tested (guideline not specified) for its mutagenic potential in Salmonella typhimurium tester strains TA 97, TA 98, TA 100, and TA 102 using the plate incorporation technique with and without S9-mix. The study consisted of two independent experiments, each conducted in the presence and absence of S9-mix. The concentrations of the test substance ranged from 0.05 to 500 µg/plate. Negative solvent control and appropriate positive controls were used in the experiments.

Results
BP-1 had significant mutagenicity on the TA 97 strain at doses of 0.05 and 0.5 µg/plate in the absence of S9-mix. However, mutation rates in the high dose experimental group decreased. No mutagenic activities were detected at any tested doses from 0.05 to 500 µg/plate in presence of S9-mix. In case of TA 100 strain, in the absence of S9-mix, mutagenic ratio (MR) was greater than 1 but less than 2 at a dose of 0.05 µg/plate. The mutation rate was more than doubled when the dose reached 0.5 µg/plate, exhibiting a significant mutation effect. However, there were no obvious mutations at the doses of 5, 50, and 500 µg/plate.
In the presence or absence of S9-mix, test substance was not mutagenic in TA98 and TA102 strains. An increasing number of inverse mutants were observed from 0.05- 50 µg/plate dose in the TA 102 strain both in presence and absence of S9-mix, but the increase was not significant. All the positive reverse mutations occurred in the absence of S9-mix.

Conclusion
Under the experimental conditions of the study, BP-1 was found to be mutagenic in the bacterial reverse mutation test in the absence of S9-mix in TA 97 and TA 100 strains while it didn't show any mutation in presence of S9-mix in all tester strains.

(Wang et al., 2018)

Applicant's Comment
There was no dose response observed in the results and mutagenicity occurred only at two lower doses in absence of metabolic activation (S9-mix). Further, this study did not follow any standard testing guidelines. Other limitations of the study included non-GLP status, no historical data and absence of well-known positive controls.

In vitro mutagenicity / genotoxicity data of analogue BP-3

The genotoxicity of BP-3 was investigated in several *in vitro* assays covering gene mutations, structural chromosome aberrations and clastogenicity in bacterial or mammalian cell systems. The available studies are summarised in Table 4.

Table 4: Overview of *in vitro* mutagenicity/genotoxicity studies with BP-3

Study type	Study details	Key results	Reference
Bacterial reverse mutation assay (OECD Test Guideline 471; GLP)	*Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 and *Escherichia coli WP2 and WP2 uvrA; S9-mix* Concentrations: 100 - 1250 µg/plate Vehicle: DMSO Positive controls: 9-Aminoacridine, 2-Aminoanthracene, Daunomycin, 1-Ethyl-2-nitro-3-nitrosoguanidine, Methyl methanesulfonate, N-	Non mutagenic with and without S9-mix	(ECHA, 2021a)

	Methyl-N-nitro-N-nitrosoguanidine, 2-Nitrofluorene, 1,2-phenylene diamine		
Mammalian Cell Gene Mutation Test (OECD Test Guideline 476; GLP)	Chinese hamster lung fibroblasts (V79), HPRT locus 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 Duration of exposure: 4 and 24 hours Vehicle: DMSO Positive controls: with S9-mix: 7,12-dimethylbenzanthracene; without S9-mix: ethylmethanesulphonate	Non mutagenic with and without S9-mix	(ECHA, 2021a)
Sister-chromatid exchanges assay	Chinese hamster ovary cells Dose: 5-50 µ g/ml) With and without S9-mix	Induced sister-chromatid exchanges in the presence of S9-mix	(French, 1992)
Chromosomal aberration assay	Chromosomal aberrations Dose: 20-45 µg/mL With and without S9-mix	Induced chromosomal aberrations in presence of S9-mix	(French, 1992)

3.4.6.2 Mutagenicity / genotoxicity *in vivo*

According to the Applicant:

No *in-vivo* mutagenicity/genotoxicity studies with BP-1 could be identified**.**

In vivo mutagenicity / genotoxicity data of analogue BP-3
The genotoxicity of BP-3 was investigated in *in vivo* assays in rodents (chromosome aberration and micronucleus test and Drosophila chromosome aberration and micronucleus test in rodents and Somatic mutation and recombination test in Drosophila. The available studies are summarised in Table 5.

Table 5: Overview of *in vivo* genotoxicity studies with BP-3

Study type	Study details	Key results	Reference
In vivo bone marrow chromosome aberration test, rats (No guideline followed)	Bone marrow chromosome aberration test Species/strain: Rats/Sprague Dawley Doses: 0, 500, 1670 and 5000 mg/kg bw/day Route: oral gavage Frequency of treatment: Single and repeated doses Vehicle: Corn oil Positive control: Cyclophosphamide	Non clastogenic	(SCCP, 2006)
In vivo micronucleus test, mice (Similar to OECD Guideline 408; GLP)	Micronucleus test Species/strain: Mice/ B6C3F1 Group size: 10/ sex/ group Doses: 0, 3125, 6250, 12500, 25000, and 50000 ppm (Equivalent to 0, 554, 1246, 2860, 6780, and 16238 mg/kg bw/day) Route: Diet Duration: 13 weeks Frequency of treatment: Daily Vehicle: No vehicle Positive control: Cyclophosphamide	Negative (No increases of micronucleated cells)	(ECHA, 2021a)
Somatic mutation and recombination test	*Drosophila* somatic mutation and recombination test (SMART) Test system: *Drosophila* melanogaster (Larvae from mated multiple wing hair females with heterozygotous flare males) Dose: 0, 3000 and 3500 ppm Duration: 72 hours	Did not induce mutations, chromosome damage or genetic recombination in *Drosophila*	(SCCP, 2006)

According to the Applicant, overall, considering the *in vitro* mutagenicity data available for BP-1 as well as *in vitro* and *in vivo* genotoxicity studies on its structural analogue BP-3, BP-1 is not considered to be genotoxic.

SCCS comment

During preliminary evaluation of the submitted dossier, the SCCS noted a number of discrepancies and shortcomings in regard to the provided evidence, and considered it to be insufficient to allow exclusion of the mutagenicity/genotoxicity potential of BP-1 on the basis that:

- the available studies on *in vitro* mutagenicity of BP-1 had either been performed with the test item of unknown purity, or were not in line with the current OECD guidelines. No study on the *in vitro* clastogenic and aneugenic potential of BP-1 had been identified.
- a number of shortcomings were noted in the study by Wang *et al*. (2018) on BP-1, e.g. details on the batch used were not given, no standard guidelines were followed, no historical controls were provided and only four of the five bacterial strains recommended by the OECD TG were tested. Despite such limitations, the study results pointed to genotoxicity of BP-1 in the bacterial reverse mutation test in the absence of S9-mix in TA 97 and TA 100 strains (while not in the presence of S9-mix).
- no historic *in vivo* studies reporting results on mutagenicity/ genotoxicity of BP-1 were available, and the submitted data pertained to the analogue BP-3. The SCCS, however, considered that the data/information on BP-3 could only be considered as supporting evidence for BP-1, when used in conjunction with other lines of evidence for BP-1.

- from the available information, the SCCS has also noted that some of the evidence was equivocal for carcinogenicity potential of BP-3. However, the need for further data to exclude carcinogenicity potential of BP-1 would depend on whether or not mutagenicity/genotoxicity potential could first be excluded.

The SCCS requested the Applicant (September 2023) to provide new experimental data for BP-1 from appropriate tests and directed the Applicant to section 3-4.10 of the SCCS Notes of Guidance (SCCS/1647/22) for details on the suite of non-animal methods that could be used to gather a weight of evidence to exclude mutagenicity/ genotoxicity of a chemical substance. In response to the SCCS request, the Applicant provided the following two *in vitro* mutagenicity studies.

***In vitro* Bacterial Reverse Mutation Test**

Guideline:	OECD Guideline 471 and EU Method B. 13/14
Species/strain:	Salmonella typhimurium (strains TA98, TA100, TA102, TA1535, TA1537)
Test substance:	Benzophenone-1
CAS no.	131-56-6
Batch:	BP1-20230305
Expiry date:	25. Mar. 2026
Stability:	Stable under storage conditions
Appearance:	Fine, white powder
Purity:	99.7 %
Homogeneity:	Homogeneous
Production date	26 Mar 2023
Concentrations tested:	up to 5000 µg/plate in different experiments (details below)
Metabolic activations:	Without and with S9 mix
Treatment duration:	24 hours at 37 ± 1 °C
Negative/ solvent controls:	DMSO, acetone, demineralized water
Positive controls:	Sodium azide, 4-nitro-1,2-phenylene diamine, benzo-a-pyrene, 2-amino-anthracene, mitomycin C
GLP:	In compliance
Study period:	27 Feb 2024 – 21 Mar 2024

According to the submitted study report, the test was performed in four valid experiments. The initial experiments 1 and 2 had to be repeated (1b, 2b) due to toxicity of the test item towards the bacteria resulting in an insufficient number of analysable concentrations as indicated in the Guideline.

Experiment 1: In the first experiment, 5 concentrations of the test item (dissolved in acetone) were tested (50; 150; 500; 150; 5000 µg/plate) in the absence and presence of S9-mix in the five strains using the plate incorporation method. The test item showed turbidity in the test mixtures at the highest concentration (5000 µg/plate) and precipitates on the plates in the experimental condition with metabolic activation at the highest tested concentration. Distinct cytotoxicity was found in all strains (for details see [Applicant's] Table 8.1-a).

No relevant or concentration-related increase of the number of revertant colonies in the treatments with and without metabolic activation could be observed at the evaluated concentrations. Based on the toxicity found in experiment 1, a repetition was performed under the same conditions (plate incorporation method, experiment 1b) with different concentrations to get enough evaluable concentrations between the toxic and the solvent range.

Experiment 1b: In the experiment 1b, 5 concentrations of the test item (dissolved in acetone) were tested (205; 256; 320; 400; 500 µg/plate for TA98, TA100, TA1535, TA1537 / 614; 768; 960; 1200; 1500 µg/plate for TA102 -S9 / 1302; 1822; 2551; 3571; 5000 µg/plate for TA102 +S9) in the five strains using the plate incorporation method.

The test item showed turbidity at the 4 highest concentrations (1822 µg/plate up to 5000 µg/plate) in the test item mixtures with S9 of TA102. Precipitates on the plates were observed at the 2 highest concentrations (5000 and 3571 µg/plate) in strain TA102 (+S9).

Distinct cytotoxicity was found in several strains (for details see [Applicant's] Table 8.2-a). No relevant or concentration-related increase of the number of revertant colonies in the treatments with and without metabolic activation could be observed at the evaluated test item concentrations. Since a negative result was obtained using the plate incorporation method (experiments 1 and 1b), a further experiment (experiment 2, pre-incubation method) was performed to verify these findings.

Experiment 2: In the second experiment, 6 concentrations of the test item (dissolved in acetone) were tested (79; 119; 178; 267; 400; 600 µg/plate for TA98, TA1535 / 53; 79; 119; 178; 267; 400 µg/plate for TA100, TA1537 / 79; 119; 178; 267; 400; 600; 900 µg/plate for TA102 -S9 / 670; 804; 965; 1157; 1389; 1667; 2000 µg/plate for TA102 +S9) in the five strains using the preincubation method.

The test item showed turbidity at the concentration 2000 µg/plate in the test mixture with S9 of TA102. No precipitates on the plates were observed at any of the tested concentrations.

Distinct cytotoxicity was found in several strains (for details see [[Applicant's] Table 8.3-a). No relevant or concentration-related increase of the number of revertant colonies in the treatments with and without metabolic activation could be observed at the evaluated concentrations.

A further experiment using the pre-incubation method was performed (experiment 2b) to get enough non-cytotoxic concentrations (TA102 +S9, TA98 +S9) or to obtain a toxic one (TA102 -S9).

Experiment 2b: In the experiment 2b, 6 concentrations of the test item (dissolved in acetone) were tested (72; 86; 103; 124; 148; 178 µg/plate for TA98 + S9 / 658; 988; 1481; 2222; 3333; 5000 µg/plate for TA102 -S9 / 500; 551; 606; 666; 733; 806 µg/plate for TA102 +S9) in the five strains using the pre-incubation method.

The test item showed turbidity in the test mixtures from 2222 µg/plate up to 5000 µg/plate of TA102 -S9. No precipitates on the plates were observed at any of the tested concentrations.

Distinct cytotoxicity was found in both tested strains (for details see [Applicant's] Table 8.4-a). No relevant or concentration-related increase of the number of revertant colonies in the treatments with and without metabolic activation could be observed at the evaluated concentrations.

Conclusion: Under the study conditions, Benzophenone-1 was not mutagenic in the Salmonella typhimurium reverse mutation assay.

Ref: Final Report: Determination of the mutagenic potential of Benzophenone-1 with the Bacterial Reverse Mutation Test following OECD Guideline 471 and EU Method B. 13/14. Study No.: 24020803G803

SCCS comment

- In **Experiment 1,** bacteriotoxicity expressed as decrease in revertant numbers was exceedingly high (even including total inhibition of bacterial growth) at the different concentrations tested. It resulted in too low a number (2-3, and 4 for TA102 +S9) of concentrations acceptable for analysis for all 5 bacterial strains. According to OECD TG 471, at least 5 valid concentrations should be tested. Interestingly, for TA102 +S9 at 1500 µg/plate, a difficult to explain, almost two-fold increase in revertants was observed (mean 605 vs. 357 in acetone negative control).
- In **Experiment 1b,** despite the use of lower concentrations of BP-1 based on Experiment 1, high bacteriotoxicity was still observed for all 5 strains. In many cases the toxicity was higher than the MIN value of the revertant number in historic acetone negative controls.

For TA102, for unknown reasons, unacceptably high concentrations were used, which based on the results from Experiment 1 should have been excluded due to bacteriotoxicity. For example, for TA102 strain tested +S9, only one (the lowest concentration at 1302 µg/plate) was acceptable for analysis.

- It could have been expected that in **Experiment 2** (preincubation test) the authors would have used carefully selected BP-1 concentrations based on previous two experiments. However, the results of the Experiment 2 again showed an unacceptably high bacteriotoxicity in all 5 strains, lowering the number of analysable concentrations to below 5. Similar to previous experiments, for TA102 strain tested +S9-mix, only one (the lowest concentration at 670 µg/plate) was acceptable for analysis.
- **Experiment 2b** (preincubation test) was repeated for TA98 +S9-mix and TA102 with or without S9 mix. However, it is not clear why TA98 was not tested without S9 mix. Again, comparison of the results at different concentrations used in this experiment with MIN revertant numbers in historical acetone negative controls indicate borderline or strong bacteriotoxicity, disqualifying the results from analysis.

For the reasons given above, the SCCS considers that this study is not valid for use in safety assessment of BP-1.

***In Vitro* Mammalian Cell Micronucleus Test**

Guideline:	OECD 487 and EU B.49
Species/strain:	Human lymphocytes
Test substance:	Benzophenone-1
CAS no.	131-56-6
Batch:	BP1-20230305
Expiry date:	25. Mar. 2026
Stability:	Stable under storage conditions
Appearance:	Fine, white powder
Purity:	99.7 %
Homogeneity:	Homogeneous
Production date	26 Mar 2023
Concentrations tested:	up to 250 µg/ mL in different experiments (details below)
Metabolic activations:	Without and with S9 mix
Treatment duration:	cell cultures were incubated at 37±1 °C in a humidified atmosphere with 5.0±0.5% CO2 for 4h
Negative/solvent controls:	0.5% acetone in culture medium; 0.5% NaCl in culture medium
Positive controls:	Cyclophosphamide monohydrate; mitomycin C; colchicine
GLP:	In compliance
Study period:	01 Mar 2024 –19 Mar 2024

According to the submitted study report, human peripheral blood lymphocytes in whole blood culture were stimulated to divide by addition of phytohaemagglutinin and exposed to solvent control, test item, and positive control. All cell cultures were set up in duplicates. After exposure and harvesting, slides were prepared and stained. In order to assess the toxicity of the test item to the cultivated human lymphocytes, the cytokinesis-block proliferation index (CBPI) was calculated for all evaluable cultures. On the basis of these data and with regard to solubility / precipitation, the ap-propriate concentrations were selected to determine the proportion of binucleated cells containing micronuclei.

The test was performed in 4 valid experiments (pre-exp., exp. I, exp. I b and exp. II). The initial pre-experiment had to be repeated (pre-exp. b = exp. I) due to toxicity of the test item towards the cultivated human lymphocytes resulting in an insufficient number of analysable concentrations as indicated in the guideline.

Pre-experiment: 5 concentrations of the test item (dissolved in acetone) were tested (0.13; 0.25; 0.5; 1; 2 mg/mL) without and with S9-mix, 4 h exposure.

The test item showed precipitation at the end of treatment (visual inspection) at the 2 highest concentrations (1 and 2 mg/mL) without S9-mix and at the 4 highest concentrations (0.25; 0.5; 1; 2 mg/mL) with S9-mix. Distinct cytotoxicity was found at all concentrations, without and with metabolic activation (for details see [Applicant's] Table 9-b).

Based on the toxicity found in the pre-experiment, a repetition was performed under the same conditions (4 h exposure, without and with S9-mix, pre-experiment b) with different concentrations to get enough evaluable concentrations between the toxic and the solvent range.

Pre-experiment b: 9 concentrations of the test item (dissolved in acetone) were tested (31; 40; 52; 67; 88; 114; 148; 192; 250 µg/mL) without and with S9-mix, 4 h exposure.

No precipitation was observed at any of the test item concentrations. Distinct cytotoxicity was found at the 3 highest concentrations (148; 192; 250 µg/mL), without and with metabolic activation (for details see chapter 9.3).

In both experimental conditions (without and with S9-mix), ≥ 3 test item concentrations could be analysed as indicated in the guideline, therefore, pre-experiment b was evaluated as experiment I. The required toxicity of 55 ± 5% was not exactly met.

In the experiment without S9-mix, 4 concentrations were evaluated for genotoxicity (40; 67; 114; 148 µg/mL) covering a range from high over moderate to no/low toxicity. The highest concentration (148 µg/mL) showed a cytotoxicity (61.1%) lying just above the required range of 55 ± 5% and was evaluated to get one concentration in a critical range of cytotoxicity. At 2 test item concentrations (67; 148 µg/mL), the ratio of micronuclei was statistically significantly increased lying only slightly above (148 µg/mL) the range (min-max and 95% control limits) of solvent controls respectively inside (67 µg/mL) the range (min-max and 95% con-trol limits; see chapter 18, historical data). No dose-dependency was observed. Since only 2 out of 3 criteria for a positive result were met, the result of experiment I without metabolic activation was assessed as "equivocal".

In the experiment with S9-mix, 3 concentrations were evaluated for genotoxicity (40; 67; 114 µg/mL) covering a range from moderate to no/low toxicity. No statistically significant increase of micronuclei was observed at any of the evaluated concentrations. All values lay inside the historical data for solvent controls (see chapter 18). No dose-dependency was observed. Since the critical range of cytotoxicity (55 ± 5% according to OECD 487) was not met, no meaningful result could be obtained and a further experiment with short exposure and metabolic activation was performed (exp. I b).

Experiment I b: 9 concentrations of the test item (dissolved in acetone) were tested (56; 64; 73; 85; 97; 112; 129; 148; 170 µg/mL) in the presence of S9-mix, 4 h exposure.

No precipitation was observed at any of the test item concentrations. Distinct cytotoxicity was found at the 2 highest concentrations (148; 170 µg/mL), for details see chapter 9.4.1. The required cytotoxicity of 55 ± 5% was met. A statistically significant increase of micronuclei was observed at the highest evaluated concentration. Due to the fact, that this value as well as all other values lay inside the historical data for solvent controls (see chapter 18) and furthermore, no dose-dependency was observed, this finding is considered as biologically not relevant. Therefore, the outcome of experiment I b is assessed as "negative".

Experiment II: 9 concentrations of the test item (dissolved in acetone) were tested (21; 27; 35; 46; 60; 77; 101; 131; 170 µg/mL) only without S9-mix, 23.5 h exposure.

No precipitation was observed at any of the test item concentrations. Distinct cytotoxicity was found at the 6 highest concentrations (46; 60; 77; 101; 131; 170 µg/mL), for details see chapter 9.5.1. The required cytotoxicity of 55 ± 5% was met. No statistically significant in-crease of micronuclei was observed at any of the evaluated concentrations. All values lay inside the historical data for solvent controls (see chapter 18). No dose-dependency was

observed. Since all criteria for a negative result were met, the outcome of experiment II is assessed as "negative".

In all experiments, the positive control compounds caused large, statistically significant increases in the proportion of binucleate cells with micronuclei, demonstrating the sensitivity of the test system. Micronucleus induction of the solvent controls was in the range of the historical control data/literature data. Therefore, the study is considered valid.

Conclusion: Under the study conditions, Benzophenone-1 does not induce the formation of micronuclei in human lymphocytes *in vitro.*

The result of the micronucleus test with the test item Benzophenone-1 is considered as "negative" under the conditions of the test.

Ref: Final Report: Determination of the potential of Benzophenone-1 to induce formation of micronuclei in human lymphocytes with the "*In Vitro* Mammalian Cell Micronucleus Test" following OECD 487 and EU B.49. Study No.: 24020803G860.

SCCS comment

- in **Experiment 1**, at the highest concentration tested, the micronucleus frequency of 1.37% is outside of 95% CI range (1.03) and MAX value of the range for negative controls (1.34), with cytostasis being not acceptable, i.e. 61.1% vs. acceptable 55±5% recommended by OECD TG 487. At the lower concentration of 67 µg/mL, there was a statistically significant increase (0.84%) in the micronucleus frequency. However, the value was within the historical control range.
  In the SCCS Opinion, the results of this experiment without S9-mix are equivocal, and to clarify the uncertainty, it should be repeated with adjustment of some parameters, such as increasing the number of binuclear cells scored. For reasons that are not clear, Experiment 1 was repeated for 4 hrs with S9-mix (for which the results were acceptable), instead of repeating the test for 4 hrs without S9-mix (for which results were equivocal in Experiment 1).
- In **Experiment 1b,** the concentrations used were not the same as in Experiment 1. There was a statistically significant increase in the micronucleus frequency at the highest concentration tested (MN frequency of 0.84% at 129 µg/mL). However, again this value was within the historical control range.

For the reasons given above, the SCCS considers that the results of this study are equivocal for 4 hr exposure to BP-1 without S9-mix.

Overall SCCS comment on mutagenicity of BP-1
Out of the two *in vitro* mutagenicity studies provided by the Applicant, the study on bacterial reverse mutation (Ames) test is considered by the SCCS as not valid, whereas the study on micronucleus induction in human lymphocytes is considered as equivocal. Taken together, the provided evidence does not allow the SCCS to conclude on the safety of BP-1 in relation to genotoxicity potential.

3.4.7 Carcinogenicity

According to the Applicant:
No carcinogenicity studies with BP-1 could be identified. The carcinogenicity endpoint has therefore been assessed on the basis of **data available on its structural analogue BP-3**.

1 **Carcinogenicity data of analogue BP-3**
2 The carcinogenic potential of BP-3 was investigated in NTP assays in rats and mice. There
3 was equivocal evidence of carcinogenic activity based on the occurrence of malignant
4 meningiomas in the brain of male rats and increased incidence of thyroid C-cell adenomas
5 and uterine stromal polyps in female rats exposed to BP-3 in the diet at concentrations up
6 to 10000 ppm (585/632 mg/kg bw/day) for 104/105 weeks. BP-3 showed no evidence of
7 carcinogenic activity in male or female mice.
8
9 An overview of carcinogenicity data available for BP-3 is presented in Table 6.
10
11
12 Table 6: Overview of carcinogenicity data available for BP-3
13

Study type, Species	Doses	Key findings	Results	Reference
Carcinogenicity study (2-year, NTP standard protocol; in utero exposure from GD 6; GLP), Sprague Dawley rats (60/sex (0 and 10000 ppm) or 50/sex (1000 and 3000 ppm)	0, 1000, 3000, 10000 ppm in diet (corresponding to 0, 58, 168 and 585 mg/kg bw/day in males and 0, 60, 180 and 632 mg/kg bw/day in females)	Malignant meningiomas# in the brain of males in 1000 and 3000 ppm groups. Incidences of C-cell adenoma in the thyroid gland, a significant increase in incidences of stromal polyp in the uterus and atypical endometrium hyperplasia of the uterus in females at 3000 ppm. Although some of the overall incidences were statistically significant, none of these neoplasms was found to be clearly exposure related. Significant increase in the incidences of focal hypertrophy in the adrenal cortex of females at 1000 and 3000 ppm. The incidence of interstitial cell hyperplasia in testes occurred with a positive trend. However, significant lack of a dose-response effect shown by these observations, with minimal changes recorded at the highest	Equivocal evidence of carcinogenicity	(SCCS, 2021a)

		tested dose compared to control.		
Carcinogenicity study (2-year, NTP standard protocol; GLP), B6C3F1/N mice (50/sex/dose)	0, 1000, 3000, 10000 ppm in diet (corresponding to 0, 113, 339 and 1207mg/kg bw/day in males and 0, 109, 320 and 1278 mg/kg mg/kg bw/day in females)	No treatment related adverse effects in organ weights including gross pathology and complete histopathology of gonads, accessory gland, thyroid and adrenal glands observed up to 10000 ppm.	No evidence of carcinogenicity	(SCCS, 2021a)

#The incidence of malignant meningiomas in the brain and spinal cord of male rats at the end of the 2-year study was 0/50, 1/50 (2%), 4/50 (8%), and 0/50 at 1, 1000, 3000 and 10000 ppm, respectively. The trend of the incidence was not statistically significant. The findings did also not show a dose-response. Since the historical control incidence for all routes of exposure of 2-year NTP studies in the rat is 0/340 (0%), the marginal increase in the incidence of malignant meningiomas in the brain of male rats, although not statistically significant, were considered to be of equivocal evidence of a marginal increase of carcinogenic activity. A dose-response relationship could not be established. Overall, there is no evidence of carcinogenicity at the lowest dose group (1000 ppm) in rats.

SCCS comment

The SCCS has noted that the Applicant has not been able to identify any carcinogenicity studies with BP-1. The studies quoted by the Applicant relate to the structural analogue (BP-3) and not BP-1. As the genotoxicity of BP-1 cannot be excluded, the SCCS is of the view that the provided evidence is also not sufficient to exclude the carcinogenicity potential of BP-1.

3.4.8 Photo-induced toxicity

3.4.8.1 Phototoxicity / photo-irritation and photosensitisation

In vitro 3T3 (NRU) phototoxicity test

Guideline: Modified OECD Guideline 432
Test system: Mice Balb/c 3T3 cells
Test substance: RI0093 (BP-1)
Batch: Not specified
Purity: Not specified
Vehicle: Hanks' Balanced Salt Solution without phenol red (HBSS) or Earle's Balanced Salt Solution without phenol red (EBSS) containing secondary solvent when appropriate Blank control

Positive control: Chlorpromazine
Exposure duration: 24 hours
Concentrations: 0.291-1000 µg/mL (Dose range study), 4.90-1000 µg/mL (Definitive assay)
Irradiation: UV-A 1.7 mW/cm2 for 50 minutes resulting in UV-A dose of 5 J/cm2
GLP: Not specified
Study year: 2010

The phototoxic potential of BP-1 was investigated in an OECD Guideline 432 *in vitro* 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT). For the assay, Balb/c 3T3 cells were sub-cultured in 96-well microtiter plates, when the culture flasks were 50-80% confluent. Prior to treatment, the culture medium (DMEM) was removed, and the cells were washed in pre-warmed Hank's Balanced Salt Solution (HBSS). Eight concentrations of the test substance were diluted from stock solution with HBSS and added to the cells. After 60 minutes of incubation, the cells were exposed to the sun simulator (Dermalight SOL 3) equipped with a UVA H1 filter (320 - 400 nm), for 50 minutes (UV-A irradiance: 1.7 mW/ cm^2, UVA dose: 5 J/cm^2). After irradiation, the cells were washed, and the uptake of Neutral red was determined 24 hours later in a plate reader at 540 550 nm (OD540 or OD550). Prediction of the phototoxic potential was achieved by calculation of the photoinhibition factor (PIF) and the mean photo effect (MPE) by comparing two equally effective cytotoxic concentrations (EC50) of the test substance obtained in the absence (-UV) and in the presence (+UV) of a noncytotoxic irradiation. The respective IC50 values were defined as the concentration of the test substance which causes a 50% reduction of NRU compared of untreated control cultures. Substances showing PIF ≥ 5 and Mean Photo Effect (MPE) ≥ 0.1 were considered phototoxic.

Results
The IC50 for the test substance was in the range of 62.8 to 79.8 µg/mL. The mean photo effect (MPE) and photo-irritancy factor (PIF) were reported to be in the range of -0.026 to 0.02 and 0.960 to 1.27 respectively. Test substance was shown to be below the respective cut-off criteria for phototoxicity with MPE <0.1 and PIF < 2.0. Chlorpromazine, the positive control, gave results indicating phototoxic potential.

Conclusion
Under the conditions of the assay, BP-1 did not show any phototoxic potential in the murine 3T3 NRU assay in the presence or absence of artificial sunlight.

(Avon Products International, 2010a)

Phototoxicity test (PT)
Guideline: Approved study protocol and standard operating procedures by the Clarus Institutional Review Board (CIRB) 2010
Species/strain: Human
Group size: 12
Test substance: BP-1 (Liquid Blend; Code)
Batch: Not specified
Purity: Not specified
Route: Dermal
Vehicle/Carrier: Not specified
Dosage levels: 0.2 g
Exposure period: 24 hours
Light source: 4 Philips F40BL fluorescent tubes
Irradiation: 4.2 ±0.4 mW/cm2 UVA radiation for approximately 17 minutes
Positive control: Not used

Observations: 24, 48 and 72 hours
Study year 2010

1% of BP-1 (Liquid Blend) was tested in a phototoxicity test using a protocol approved by the CIRB in 12 human volunteers. A Webril/adhesive patch was used occlusively. Approximately 0.2 g of the test substance was applied to each patch for 24 hours to the subject's back for a 24-hour. The test site was then irradiated by UV-A irradiation at a dose of 4.2 ±0.4 mW/cm2 for approximately 17 minutes by using 4 Philips F40BL fluorescent tubes. As the dosage of Ultraviolet-A (UVA) irradiation is not erythrogenic, no control site was delineated on the subject's back rather, the entire back served as an irradiated control. The non-irradiated test site was placed on the opposite side of the subject's back.
Approximately after 24, 48 and 72 hours post-patching, patches were removed from the test site to be irradiated and scored using the modified scoring scale of the International Contact Dermatitis Research Group System. The subject's nonirradiated test site was protected from the light source by the subject's own clothing or by the patch. After removal, the application sites were scored at 24, 48, 72 hours following exposure of the site to UV radiation for degree of irritation and sensitisation.

Results
All 12 volunteers completed the study. No serious adverse events occurred during this test. No reactions were exhibited on either the irradiated or the non-irradiated test substance contact sites. No reactions were exhibited on the irradiated (without test substance) control site.

Conclusion
Under the conditions of the study, BP-1 did not induce a dermal phototoxic response in any of the 12 panellists completing the study.

(Avon Products International, 2010b)

<u>Photoallergenicity test</u>

Guideline:	Approved study protocol and standard operating procedures by the Clarus Institutional Review Board (CIRB) 2010
Species/strain:	Human
Group size:	30 subjects were enrolled; 29 subjects completed the test
Test substance:	BP-1 (Liquid Blend)
Batch:	Not specified
Purity:	Not specified
Route:	Dermal
Vehicle/Carrier:	Not specified
Dosage levels:	0.2 g
Exposure period:	Induction: Twice a week, a total of six induction patches
Light source:	4 Philips F40BL fluorescent tubes
Irradiation:	UVA radiation: 4.2 ±0.4 mW/cm2 for approximately 17 minutes
UVB radiation:	1.4 ±0.4 mW/cm2
Positive control:	Not used
Observations:	48, 72 and 96 hours
Study year:	2010

1% BP-1 (Liquid Blend) was tested in photoallergenicity test using a protocol approved by the CIRB in 29 human volunteers. Webril/adhesive patches containing 0.2 g of test substance was applied for 24 hours occlusively to the subject’s back, 3 times per week for 3 consecutive

weeks and additional site served as the irradiated (without test substance) control site. The designated test site was irradiated with both Ultraviolet-B (UV-B) and Ultraviolet-A (UV-A). The test site was irradiated by UV-A irradiation at a dose of 4.2 ±0.4 mW/cm2 for approximately 17 minutes respectively using 4 Philips F40BL fluorescent tubes and UVB radiation was irradiated 1.4 ±0.4 mW/cm2.
After two weeks of rest period, a challenge patch was applied to the virgin sites to each of the study participants for 24 hours using the UV filter concentrations in the same test substance formulation.
Approximately after 24 hours post-patching, patches were removed from the test site to be irradiated and scored using the modified scoring scale of the International Contact Dermatitis Research Group System. The subject's nonirradiated test site was protected from the light source by the subject's own clothing or by the patch. After removal, the application sites were scored at 24, 48, 72 and 96 hours following exposure of the site to UV radiation for degree of irritation and sensitisation.

Results
29 subjects completed the test. One subject discontinued due to personal reasons. No subject discontinued due to test substance reaction.
Test site: During the Induction Phase, low-level (±/1) reactions were exhibited on the irradiated test site. No reactions were exhibited on the nonirradiated test site. Low-level (±/1) reactions were exhibited on the irradiated (without test substance).
Control site. During the Induction Phase, the irradiated sites (with and without test substance) were observed to have slight tanning responses. During the Challenge, one subject exhibited low-level (±/1) reactions on both the irradiated and the non-irradiated test sites. No reactions were exhibited on the irradiated (without test substance) site.

Conclusion
Under the conditions of the study, BP-1 did not show photoallergic or dermal sensitisation potential in any of 29 panellists completing the study.

(Avon Products International, 2010c)

SCCS comment
The test results from the two studies in humans have limited value for use in safety assessment, whereas the *in-vitro* 3T3 NRU study indicates absence of phototoxic potential. The SCCS agrees that BP-1 is not likely to be phototoxic.

3.4.8.2 Photomutagenicity / photoclastogenicity

According to the Applicant, except for a photomutagenicity tests for which only limited information on methodologies and findings is available, no photomutagenicity / photoclastogenicity studies could be identified for BP-1 in the literature. Therefore, data on the analogue BP-3 were used for assessing these endpoints.

Data on BP-1

BP-1 was tested for photomutagenicity in a Luminiscent umu test with S. typhimurium strain TL210 using luciferase gene and light absorption umu-test using S. typhimurium TA1535/pSK1002 strain respectively. The concentrations of the test substance ranged from 0 to 10 µg/well. DMSO and methanol were used as dilution solvents. BP-1 showed a pseudo positive response in the luminescent, and it produced only small dose dependent increase in umu activity in presence of methanol as the solvent.

Comment: Since the sensitivity is generally considered higher in the luminescent umu-test than the light absorption version, the author confirmed that the difference in sensitivity is believed to be the reason why the compounds that showed positive or pseudo-positive responses in the luminescent umu-test produced negative results in the light absorption test.

(Nakajima et al., 2006)

The photo-genotoxicity of BP-1 (1 to 25 µg/mL, in culture medium) and apoptotic parameters were evaluated using human keratinocytes (HaCaT cells). Cells exposed to different concentrations of BP-1 in the presence of UVA (2.7 J/cm2) exhibited statistically significant ($p > 0.01$) DNA damage when compared to control cells. Under the test conditions, BP-1 photosensitised and generated reactive oxygen species in the presence of sunlight/UV radiation.

In an *in vitro* genotoxicity test, human keratinocytes (HaCaT) cells were exposed to BP-1 at 10 µg/mL in the presence of UVB (1.08 J/cm2). Micronuclei formation was detected in HaCaT cells when radiated with UVB.

(CIR, 2021)

Data on the structural analogue BP-3

The photomutagenicity/photoclastogenicity of analogue BP-3 was investigated in various OECD Test Guideline studies. The available studies are summarised in Table 7.

Table 7: Overview of photomutagenicity/photoclastogenicity studies with BP-3

Study type	Study details	Key results	Reference
Bacterial reverse mutation assay (OECD Guideline 471; GLP)	*Salmonella typhimurium* strains TA 1537, TA 98, TA 100 and TA 102 Concentrations: 3- 5000 µg/plate Experiment 1: 3- 5000 µg/plate Experiment 2: 10- 5000 µg/plate Vehicle: DMSO Irradiation: Artificial sunlight Positive controls: Not specified	Non photomutagenic with and without S9-mix	(SCCP, 2006)
Chromosomal aberrations Test (OECD Guideline 473; GLP)	Chinese hamster lung fibroblasts (V79), HPRT locus Concentrations: 8.92 – 2283 µg/mL Experiment 1: 3.1, 6.3 12.5, 25, 50, 75 µg/mL; 225 mJ/cm2 UVA Experiment 2: 3.1, 6.3 12.5, 25, 50, 75 µg/mL; 225 and 375 mJ/cm2 UVA Duration of exposure: 3 hours Vehicle: DMSO Light source: Xenon burner with an additional special filter glass, emitting visible light and UVA/UVB light (ratio: about 30:1) > 290 nm was used as light source. Positive controls: with S9-mix: 8-Methoxypsoralene; without S9-mix: Ethylmethane sulphonate	Non photoclastogenic with and without S9-mix	(SCCP, 2006)

According to the Applicant: Overall, based on the available *in vitro* data with BP-1 and BP-3, BP-1 is not expected to induce photomutagenicity or photoclastogenicity.

SCCS comment

Based on the submitted information and evaluation of the source document (Amar *et al*., 2015) of the CIR 2021 report, the SCCS is of the view that there is insufficient evidence for photomutagenicity. In the absence of phototoxicity, the SCCS considers that photomutagenicity of BP-1 is also unlikely.

3.4.9 Human data

The Applicant referred to sections on Toxicokinetics, skin irritation, skin sensitisation, and phototoxicity.

Skin irritation

Study 1: occlusive patch test:
In a single patch application study, BP-1 was applied at concentrations of 4, 8 and 16% in DMP or petrolatum to 14 panellists. Under the test conditions, BP-1 was non-irritating up to the highest tested concentration in both vehicles. No further study details are available.

Single insult patch test - splash cologne formulation containing BP-1

Guideline:	Not available
Species/strain:	Human
Group size:	18
Test substance:	Formulation containing BP-1
Batch:	Not specified
Route:	Dermal
Administration:	Occlusive
Exposure period:	24 hours
Applied Dose:	1%
Application frequency:	Once
Concentration:	100%
Scoring system:	Primary Irritation Index (PII)
Study period:	Not specified

BP-1 was evaluated in a single insult patch test (SIPT) in 18 human volunteers. Subjects were exposed to a splash cologne containing BP-1 at 1%. The test substance was applied occlusively to a small area (approximately 2 x 2 cm2) for 24 hours. The irritation reactions were provided as the average Primary Irritation Index score.

Results
No signs of irritation were observed. The Primary Irritation Index was 0.00.

Conclusion
Under the conditions of the SIPT, no reactions indicative of a skin irritation response was observed in any of 18 panellists completing the study.

(Harrington, 2010) [reference for this unpublished study is of 2011]

Study 2: Single insult patch test - body mist formulation containing BP-1

Guideline:	Not available
Species/strain:	Human
Group size:	21
Test substance:	Formulation containing BP-1
Batch:	Not specified
Route:	Dermal
Administration:	Occlusive
Exposure period:	24 hours
Applied Dose:	0.5%
Application frequency:	Once
Concentration:	100%
Scoring system:	Primary Irritation Index (PII)
Study period:	Not specified

BP-1 was tested in a single insult patch test (SIPT) in 21 human volunteers. Subjects were exposed to a body mist formulation containing BP-1 at 0.5%. The test substance was applied occlusively to a small area (approximately 2 x 2 cm2) for 24 hours. Reactions were then evaluated for degree of irritation expressed as an average Primary Irritation Index score.

Results
No signs of irritation were observed. The Primary Irritation Index was 0.00.

Conclusion
Under the conditions of the SIPT, no skin reactions indicative of a skin irritation response was observed in any of 21 panellists completing the study.

(Harrington, 2007)

Skin sensitisation

Study 1: Human repeated insult patch test (HRIPT)

Guideline:	Not available
Species/strain:	Human
Group size:	100 volunteers
Test substance:	BP-1
Batch:	Not specified
Route:	Dermal
Administration:	Not specified
Application frequency:	Alternate days
Concentration:	1% in butyl carbitol
Scoring system:	Not specified
Study period:	1952

BP-1 was tested in a Shelanski human repeat insult patch test (HRIPT) in 100 human volunteers. The substance was applied to patch sites of the subjects at a concentration of 1% in butyl carbitol for 10 alternate days. After a 7-day rest period, challenge patches were applied. No further study details are available.

Results
No signs of irritation or sensitisation were observed in any of the treated subjects

Conclusion
Under the conditions of the HRIPT, no skin reactions indicative of a skin sensitisation response were observed in any of the subjects.

(CIR, 1983)

Study 2: Human Kligman maximization allergy test – nail enamel formulation containing 0.4935% BP-1

Guideline:	Not available
Species/strain:	Human
Group size:	28 volunteers
Test substance:	Formulation containing BP-1
Batch:	Not specified
Route:	Dermal
Administration:	Occlusive
Exposure period:	48-hour exposure, 10-14 days later followed with a 48-hour challenge
Applied Dose:	0.4935%
Application frequency:	Alternate days

No of applications:	5
Scoring system:	Not specified
Study period:	Not specified

BP-1 was tested in a Kligman maximization test in 28 human volunteers. Prior to exposure to the substance, panellists were treated with Sodium Lauryl Sulfate (SLS) under a patch for 24 hours to slightly impair the skin barrier function maximizing the potential for dermal penetration and potential for inducing sensitisation of the cosmetic ingredients. Subjects were subsequently exposed to the nail enamel formulation containing 0.4935% BP-1 in 15 mm Webril disc for a 48-hours under occlusion. The application areas were then evaluated for signs of irritation or sensitisation. The sequence of 24-hour SLS pre-treatment followed by 48 hours of test substance application was continued for a total of 5 induction periods. After the induction phase, panellists were tested 10-14 days later with a 48-hour challenge followed by evaluation for potential sensitisation.

Results
No signs of irritation or sensitisation were observed during any phase of the study.

Conclusion
Under the conditions of the Kligman maximization test, no reactions indicative of a skin sensitisation response was observed in any of 28 panellists completing the study.

(Avon products International, 1996)

Study 3: Human repeated insult patch test (HRIPT) Cologne and body mist formulations containing 1% BP-1

Guideline:	Not available
Species/strain:	Human
Group size:	103-113 volunteers
Test substance:	Formulations containing BP-1
Batch:	Not specified
Route:	Dermal
Administration:	Occlusive
Exposure period:	Induction: 24 hours Rest: 10-15 days Challenge: 24,48 and 72 hours
Applied Dose:	1% in 3 different formulations
Application frequency:	Induction: 9 consecutive applications
Concentration:	Not specified
Scoring system:	Not specified
Study period:	Not specified

A human repeated insult patch test (HRIPT) was performed with 3 different formulations containing 1% BP-1 in a panel of 103-113 male and female volunteers. Two colognes and one body mist formulation containing 1% BP-1 was applied occlusively over a 6-week period involving 3 phases: (1) induction, (2) rest and (3) challenge. The induction phase consisted of 9 consecutive applications of the test substance under occlusion for 24 hours and subsequent evaluations of the patch sites. Ten to fourteen days after removal of the last induction patches, a challenge patch was applied to each of the study participants for 24 hours in the same manner to fresh sites. The sites were graded after 24, 48 and 72 hours of application.

Results
No signs of irritation or sensitisation due to the test substance were observed.

Conclusion
Under the conditions of the HRIPT, no skin reactions indicative of a skin sensitisation response was observed in any of panellists completing the study.

(Avon Products International, 2001; 2015; 2019)

Toxicity data of analogue BP-3
The skin sensitising potential of BP-3 has been evaluated in GLP compliant OECD Test Guideline Local Lymph Node (LLNA) and Guinea Pig Maximisation (GPMT) assays as well as in a modified Draize Shelanski human repeat insult patch test (HRIPT). Based on the results of these studies, BP-3 is not expected to cause skin sensitisation.

Table 8: Overview of photomutagenicity/photoclastogenicity studies with BP-3

Study type	Study details	Key results	Reference
Local Lymph Node assay (LLNA) (OECD Guideline 429; GLP)	Test system: Mice / CBA-CaOlaHsd Dosage levels: 0, 12.5, 25, 50% (w/v) Group size: 4 animals per group (female) Vehicle: Dimethylformamide (DMF)	Non sensitiser Stimulation indices (SI) of 1.64, 1.33 and 1.61 were determined at concentrations of 12.5, 25 and 50% (w/v) in DMF, respectively.	(ECHA, 2021b)
Guinea Pig Maximisation assays (GPMT) using Magnusson Kligman Maximisation protocol (OECD Guideline 406; GLP)	Test system: Guinea pig/ Pirbright White Strain (Tif: DHP) Group size: 10 (test group); 5 (control group) Intradermal induction: 5 and 1 % in Oleum arachidis Epicutaneous induction: 1, 5, 10, and 30% in Vaseline Challenge: 0.5 mL of undiluted test substance (epicutaneous, occlusive) Vehicle (for intradermal): Paraffin oil or Freund's Complete Adjuvant (FCA) / 0.9% aqueous NaCl solution (1:1) Route: Dermal Administration: Intradermal and Topical	Non sensitiser At the 24- and 48-hour readings after challenge, only 1 animal out of 20 showed weak skin reactions. The other animals did not show oedema or irritation.	(ECHA, 2021b)

	Vehicle: Oleum arachidis (for intradermal) and Vaseline® (for topical) Positive control: 1-Chlor-2,4-dinitrobenzol		
Human repeated insult patch test (HRIPT)- modified Draize Shelanski method	Species: Human Group size: 100 volunteers Route: Dermal Administration: Occlusive epicutaneous Exposure period: 24 hours Applied Dose: 300 mg Application frequency: Alternate days Concentration: 25% in petrolatum	Non sensitiser No signs of irritation or sensitisation due to the test substance application	(SCCP, 2006)

SCCS comments

The Applicant gave the wrong title to Table 7 above, whichindicated that it concerned the photomutagenicity/ photoclastogenicity of BP-3, whereas the information provided in this Table relates to skin sensitisation potential. The SCCS considers studies based on HRIPT to be unethical. However, the SCCS has noted that the available information indicates that BP-1 is not a skin sensitiser.

3.4.10 Special investigations

BP-1 was tested in a Bhas assay for carcinogenic promotion using sub confluent Bhas42 cells. The cell suspension was divided between a 6-cell microplate at 2 mL/well for the promotion test and a 24 well microplate at 0.5 mL/well for the cytotoxicity test and incubated for 3 days. On the third day of incubation, the medium was removed, and replaced by the medium containing the test substance BP-1. Based on the results of the dosage test, six doses consisting of 2, 5, 10, 20, 50 and 100 µg/mL were used for the main test. The light absorbance at 540 nm was measured using Sunrise Classic (Wako Pure Chemical Ind.) for the calculation of survival rates. In the BP-1 group, there was no increase in the number of foci at concentrations below 5µg/mL. however, at 10 µg/mL, there was a significant increase to 6.0 ± 2,4 foci/well, which was more than twice that of the number of foci in the solvent controls (2.2 ± 1.5 foci/well). The increase was 1.5%/ gram when compared to the number of foci in the positive controls (50 ng/mL TPA, 20.2 ± 5.2 foci/well). At the highest concentration of 20 µg/mL, the number of foci was comparable to that of the solvent controls, but the cell survival rate was lower (31%), suggesting the toxicity of the test substance. Based on these results, which satisfied one of the positive response criteria of "*a significant increase to more than twice that of the controls*". BP-1 was believed to be a tumour promoter at 10 µg/mL. However, its potential was apparently weak in comparison to the level in the positive controls. Based

1 on the results, the study authors indicate that BP-1 did not result in a statistically significant
2 increase in the number of foci (relative to the solvent controls DMSO and methanol) over the
3 range of concentrations tested, indicating negative promotion activity. Further, this assay was
4 reported to have a high rate of false positives.
5 (Nakajima *et al.*, 2006)
6
7 **Endocrine disruption (ED) properties**

8 **Assessment of the evidence**

9 The World Health Organisation (WHO) defines an endocrine disruptor as an exogenous
10 substance or mixture that alters function(s) of the endocrine system and consequently causes
11 adverse health effects in an intact organism, or its progeny, or (sub)populations (WHO/IPCS,
12 2002).

13 The strategy to identify the endocrine disrupting properties of BP-1 includes the gathering
14 and collation of ED relevant information; the assessment of all evidence; a mode of action
15 analysis if deemed necessary and subsequently a safety evaluation ([Applicant's] **Chapter**
16 **3.5**).

17

18 **Gathering and collation of ED relevant information**

19 Relevant data for the assessment of the potential endocrine disruption (ED) properties of BP-
20 1 and its analogue BP-3 are available in the form of *in vitro* and *in vivo* studies. In addition,
21 the US EPA ToxCast database was consulted for existing high throughput screening (HTS)
22 information.

23 The endocrine assessment of the read across substance BP-3 has been recently performed by
24 SCCS in March-2021. For completeness, in the current dossier, the overview of available *in*
25 *vivo* OECD level 4 ED studies which are critical for the assessment of BP-1 are presented in
26 **Annex II**. Further details are also available in **Sections 3.3.5 and 3.3.6**.

27 The following table summarises the relevant scientific information for the assessment of the
28 ED properties of **BP-1.** The studies have been organised according to the levels described in
29 the OECD "Conceptual Framework for testing and assessment of endocrine disrupters" (OECD,
30 2018).

31 Detailed robust study summaries of data which are critical for the ED assessment (including
32 Level 4 studies) are presented in **Section 3.3** of this dossier.

Table 9: Summary of the available evidence used for the ED assessment of BP-1

Level 1: Existing data and non-test information (not related to a specific receptor): No existing data and non-test information for BP-1 were identified

Level 2: *In vitro* mechanistic assays

Studies available	Cell line/species doses/duration	Results	Reference/Klimisch (KL) scoring
In vitro high throughput screening (HTS) assays	Various human and rat cell lines/ from 0.0005 to 200 µM / 0.5 to 80 hours	BP-1 was found to be active in 26 (E=16; A=7; T=2; S=1) out of 42 ED relevant assays. However, 5 (19%) have been flagged as	CompTox/EDSP-21 (accessed in August 2021)

		potentially 'false positive' by the automated analysis tool from the US EPA.	
In vitro estrogen receptor binding assay -	Uterine cytosol (estrogen receptor) preparation /	The fifty percent inhibitory value (IC50) value of the test substance was 36.5 µM, while E2 displayed an IC50 of approximately 8.99x10^{-4} µM. Receptor	(Blair et al., 2000)/ KL-2
estrogenic activity	10-5 - 1000 µM / 20 hours	binding affinity of BP-1 was 0.002 (% of EE).	
In vitro estrogen receptor binding assay - estrogenic activity	Fluorescein-labelled 17β-estradiol / 5 × 10-10 to 5 × 10-4 M (i.e., 5 × 10-4 to 500 µM)	IC50 of BP-1 was 5 × 10-5 M (50 µM)	(Nakagawa and Suzuki, 2002)/ KL-4
In vitro estrogen receptor binding assay - estrogenic activity	HERα and HERβ competitive binding assay / 60 mins	IC50, relative binding affinity (RBA) values for ERα and Erβ were 86 µM, 44 and 2.2 µM, 8.6, respectively.	(Mutsumoto et al., 2005)/ KL-2
In vitro cell proliferation assay - estrogenic activity	Human breast cancer cell line (MCF-7) / 0.001-500 µM / 5 days	Cell numbers were increased in a concentration dependent manner from 0.01 µM to 1 µM. Cytotoxicity reported at greater than 10 µM.	(Nakagawa and Suzuki, 2002) / KL-2
In vitro yeast hER binding assay - estrogenicity assay	Yeast cells carrying the pGBT9-estrogen receptor ligand binding domain and pGAD424-TIF2 plasmids / 4 hours	The concentration of E2 showing 10% of the 1.0 X 10-7 M activity (0.1 µM) (relative effective concentration, REC10) was 1.7 X 10-10 M (1.7 X 10-4 µM). REC10 value for BP-1 was 6.5 X 10-7 M (0.65 µM). BP-1 can be considered to be 1000 times less potent than E2.	(Takatori et al., 2003)/ KL-2
In vitro E. coli. hER binding assay	Recombinant human estrogen receptor ligand binding domain (hERα-LBD) fused with glutathione S-transferase and	Receptor binding affinity of BP-1 was 0.0139 (% of EE).	(Yamasaki et al., 2004)/ KL-4

	expressed in E. coli. / 10-5 – 100 µM / 1 hour		
In vitro cell proliferation assay - estrogenic activity	MCF-7 cells / 10-4 to 10-7 M (i.e., 0.1 to 100 µM)/ 24 hours	BP-1 reported an EC50 of 2.08 µM. An EC50 value for E2 was 1.22 pM (1.2×10-6 µM). Based on the EC50 values, BP-1 estrogenic activity was 6 times less potent than E2.	(Schlumpf and Lichtensteiger, 2001 [the actual reference is Schlumpf et al., 2001]; Schlumpf et al., 2004)/ KL-2
In vitro ERE luciferase reporter gene expression assay- Estrogenic activity	MCF-7 / 0.01-100 µM / 24 hours	BP-1 showed estrogenic activity with an EC50 value of 1.26 µM. Result for control groups were not reported.	(Suzuki et al., 2005) / KL-2
In vitro cell proliferation assay - estrogenic activity	MCF-7 and Chinese hamster ovary (CHO) cell transformed with the gene encoding the human estrogen receptor (ER α) and an estrogen responsive promoter linked to a reporter gene	REC10 / relative activity (RA) values for MCF-7 assay and CHOOSER assay were 1.2 µM / 4.2 and 15 µM / 35, respectively.	(Matsumoto et al., 2005) / KL-2
In vitro hERα binding assay - estrogenic activity	Chinese hamster ovary cell line (CHO-K1) / 10-4 –10-10 M (i.e., 102 to 10-4 µM) / 16-24 hours	BP-1 reported EC50 and PC50 values of 1.5×10-6 M and 7.7×10-7 M (1.5 µM and 0.77 µM), respectively. BP-1 showed approximately 1/100000 of the E2 activity.	(Kawamura et al., 2005) / KL-2
In vitro hERα reporter gene assay – (anti)estrogenic activity	hERα ligand screening system and Recombinant yeast cells (Saccharomyces cerevisiae) that express hERα with the β-galactosidase reporter gene / 1 mM (103 µM)/ 1 hour for ELISA and 4 hours for modified yeast two-hybrid assay	ER ELISA: BP-1 showed relative binding affinity to Diethylstilbestrol values of 1.4 and 2.4 without and with S9-mix, respectively. Yeast two-hybrid assay: In agonist assay 1 mM (103 µM) BP-1 showed relative activity (RA) to E2 values of 0.021 and 0.0052 without and	(Morohoshi et al., 2005)/ KL-2

		with S9-mix, which confirmed that BP-1 had weak agonistic effects without S9-mix. In antagonist assay, BP-1 reported EC50 values of >5000 nM (5 µM) without and with S9-mix, which confirmed that BP-1 had no antagonistic effects.	
Studies available	Cell line/species doses/duration	Results	Reference/Klimisch (KL) scoring
In vitro, yeast rtERα and hERα transactivation assay/ estrogenic activity	Recombinant yeast carrying the estrogen receptor of rainbow trout (rtER α) and human estrogen alpha (hER α)	BP-1 had maximal responses of 114% compared to E2 in the rtERα assay and IC50 value of 79.9 µM (87 times less potent than E2). BP-1 showed a maximal response of 96% in the hERα assay with an IC50 of 1.15 µM (5000 times less potent than E2). The activity of BP-1 was relatively higher with rtERα than with hERα.	(Kunz et al., 2006) / KL-2
In vitro, yeast hERα transactivation assay/ (anti)estrogenic activity	Recombinant yeast carrying the human estrogen alpha (hER α)	BP-1 reported agonist EC50 value of 1.15 µM with 96% efficacy (5000 times less potent than E2). No antiestrogenic effects were observed.	(Kunz and Fent, 2006)/ KL-2

In vitro E. coli. hER binding assay	Recombinant human estrogen receptor ligand binding domain (hERα-LBD) fused with glutathione S-transferase and expressed in E. coli. / 10-5 - 100 µM / 1 hour	Receptor binding affinity of BP-1 was 0.0138 (% of EE).	(Akahori et al., 2008) / KL-2
In vitro, binding/luciferase reporter gene expression/cell proliferation assays-estrogenic activity	MCF-7-ERE-Luciferase-Neo (MELN) and HeLa-ERE-Luciferase-Neo (HELN) cells for estrogenicity / 0.01–10 µM / 3 hours	BP-1 showed non-specific induction of luciferase expression in MELN and HELN cells with respective EC50 values at 9.19 and 30 µM, which were >106-fold higher compared to E2 (1.4x10-5 and 1.9 x 10-5 µM). In whole cell competitive binding assays, BP-1 inhibited the binding of [3H]-E2 toward these receptors in a concentration-dependent and competitive manner. BP-1 inhibited proliferation in a clear dose-dependent manner when BP derivatives were applied to HELN Erα and -Erβ cells. Overall, in this test system BP-1 was concluded to have specific estrogenic activity.	(Molina-Molina et al., 2008)/ KL-2
In vitro luciferase reporter gene expression assay-estrogenic activity	Zebrafish liver cell line (ZFL) transfected with ERE-luciferase construct / 72 hours	E2 had higher potency on zfERβ1 and zfERβ2 than on zfERα, with EC50 of 0.027 nM (2.7 x 10-5 µM), 0.051 nM (5.1 x 10-5 µM), and 0.20 nM (20 x 10-5 µM), respectively. EC50 and relative estrogenic potencies values for BP-1 in zfERβ2 and zfERα were 3859 nM (3.859 µM) &	(Cosnefroy et al., 2012)/ KL-2

		1.3 x 10-5, 2195 nM (2.195 µM) and 9.3 x 10-5, respectively. BP-1 was reported to have slightly higher affinity for the zfERα than for the beta subtypes.	

In vitro cell viability assay – estrogenic activity	BG-1 human ovarian cancer cells / 0.01 to 10 µM	BP-1 (0.01 to 10 µM) statistically significantly increased BG-1 cell growth, as did E2. Both BP-1 and E2 induced cell growth and up-regulation of cyclin D1 were reversed by co-treatment with an ER antagonist, suggesting that BP-1 may, similar to E2, mediate the cancer cell proliferation via an estrogen receptor-dependent pathway. However, the expression of p21 (regulator of cell cycle progression at G1 phase) was not altered by BP-1, though it was down-regulated by E2.	(Park et al., 2013)/ KL-3
In vitro cell proliferation assay – estrogenic activity	MCF-7 human breast cancer cells / 0.01 to 10 µM	Treatment of the cells with BP-1 (0.01 to 10 µM) promoted the proliferation and migration of MCF-7 cells in a manner that was similar to E2. On treatment of the cells with BP-1 (10 µM) in the presence of an ER antagonist, BP-1 induced growth of MCF-7 cells was restored to level of a	(In Sol-Ji et al., 2015)/ KL-3

		control, indicating that BP-1 may promote proliferation of breast cancer cells through an Erα-dependent pathway.	
In vitro, cell proliferation assay – Estrogenic activity	MCF-7 / 10-5 M (i.e., 0.1 μM) / 24–25 weeks	BP-1 showed increased migration following exposure in both estrogen responsive (MCF-7) and estrogen unresponsive (MDA-MB-231) human breast cancer cells, implying that their ability to increase cell motility was not confined to estrogen-responsive cells.	(Alamer and Darbre, 2018)/ KL-2
In vitro binding and luciferase reporter gene assay- estrogenic activity	ERRγ LBD and ERRγ transfected HepG2 cells / 102-106 nM (0.1 to 103 μM) / 10 min and 48 hours	BP-1 exhibited relatively weak binding affinity with ERRγ-LBD, with the IC50 127.9 μM and relative binding affinity (RBA) value of 5.6. BP-1 showed transcriptional activity enhancement with the lowest observed effective concentration (LOEC) of 3.12 μM.	(Zheng et al., 2020)/ KL-2
In vitro ARE reporter gene assay - (anti)androgenic activity	Rat fibroblast (NIH3T3) cell line / 10-5 to 10-8 M (0.01 to 10 μM)	BP-1 showed inhibitory effect on the dihydrotestosterone (DHT)-induced AR activity, with an IC50 value of 10 μM.	(Suzuki et al., 2005)/ KL-2
In vitro hAR binding assay - (anti)androgenic activity	Chinese hamster ovary cell line (CHO-K1) / 10-4 –10-10 M (i.e., 102 to 10-4 μM) / 16-24 hours	The androgenic activity could not be detectable at <10-4 M (<100 μM). BP-1 reported EC50 and PC50 values of >1×10-4 M (>100 μM) and 1.8×10-5 M (18 μM), respectively.	(Kawamura et al., 2005)/ KL-2
In vitro, yeast hAR transactivation assay/	Recombinant yeast carrying the human	No androgenic effects were observed. BP-1 showed anti-	(Kunz and Fent, 2006)/ KL-2

(anti)androgenic activity	estrogen alpha (hER α) / 10 µM	androgenic activities with IC50 value at 0.69 µM which was about 4 times less potent compared to the flutamide with an IC50 value of 4.32 µM.	
In vitro antiandrogenic activity	HEK-293 cells transiently expressing human 17β-hydroxysteroid dehydrogenase type 3 (HSD3) / 20 µM / 45 min	BP-1 concentration-dependently inhibited 17β-HSD3 (IC50 1.05 µM) and therefore testosterone formation.	(Nashev et al., 2010)/ KL-3
Ex vivo antiandrogenic activity	Freshly isolated tissue from decapsulated testes of mice and rats / 15 min	Conversion of androgen to testosterone in mouse testis tissue was inhibited by more than 80% at BP-1 concentrations of 5 µM and higher. Similar observations were made in assays with rat testis tissue.	(Nashev et al., 2010)/ KL-2
In vitro hAR reporter-gene transactivation assay – antiandrogenic activity	HEK-293 cells / 24 hours	BP-1 inhibited testosterone-dependent AR activation with IC50 value of 5.7 µM (about five times lower than that for the AR).	(Nashev et al., 2010) / KL-2
In vitro cell proliferation and migration assay / (anti)androgenic activity	LNCaP Pca cells/ 10−5-10−8 M (i.e., 10 to 0.01 µM) / 4-6 days	Results suggested that the proliferative and migration effects of BP-1 on LNCaP cells was mediated by the androgen receptor signalling pathway	(Kim et al., 2015)/ KL-3
In vitro gene expression assay / thyroid activity	Rat pituitary (GH3) and thyroid follicle (FRTL-5) cell lines / 0.2-6.9 mg/L with GH3 cell line and 2.1-68.6 mg/L with FRTL-5 cell line	Significant down-regulation of the Tshβ, Trhr, and Trβ genes at doses of 10 µM and below. Nis gene was significantly up-regulated. Non-statistically significant change in Tg gene transcription. BP-1 did not affect the transcription level of	(Lee et al., 2018)/ KL-2

		the Tshr gene but significantly down-regulated the Tpo gene.	
In vitro steroidogenesis activity	H295R cells / 1-10 µM / 48 hours	BP-1 did not produce 1.5 fold changes in steroids progestins, adrenal androgens, mineralocorticoids, glucocorticoids.	(Strajhar et al., 2017)/ KL-2

Level 3: *In vivo* mechanistic assays

Uterotrophic assay (OECD Guideline 440; non-GLP) – (anti)Estrogenic activity	Mice / 0, 30, 100, 300 and 1000 mg/kg bw/day oral and SC / 7 days	BP-1 showed estrogenic activity by oral route and both estrogenic and anti-estrogenic activity by subcutaneous (SC) exposure at 1000 mg/kg bw/day in mice. As a result, author reported LOAEL values were 1000 mg/kg bw/day for oral agonist activity and 300 mg/kg bw/day for SC agonist and antagonist activity. The NOAEL can be established at 300 mg/kg bw/day for oral agonist activity and 100 mg/kg bw/day for SC agonist and antagonist activity.	(Ohta et al., 2012)/ KL-2
Uterotrophic assay – (anti)Estrogenic activity	Rats/ 0, 100, 250 and 625 mg/kg bw/day SC / 3 days	ED10 and ED50 values of BP-1 were 590 (wet uterine weight), 540 (blotted uterine weight) and 650 (wet uterine weight), 630 (blotted uterine weight) mg/kg bw/day, respectively. The relative estrogenic potency of BP-1 against EE was estimated to be about 1/10,000,000 when	(Koda et al., 2005)/ KL-2

		calculated using the ED10 for blotted weight	
Uterotrophic assay (Comparable to	Rats/ 0, 100, 300 and 1000 mg/kg bw/day	BP-1 showed dose dependant increase in uterine weight in all treated groups indicating estrogenic response	(Yamasaki et al., 2004)/ KL-2
OECD Guideline 440; GLP) – (anti)Estrogenic activity		SC / 3 days	
Uterotrophic assay (Similar to OECD Guideline 440; non-GLP) – Estrogenic activity	Rats/ 2-1200 mg/kg bw/day oral / 3 days	BP-1 showed increase in uterine weight in rats fed via diet, indicating weak estrogenic response. No more details about NOAEL/LOAEL were reported in the study.	(Schlumpf et al., 2004)/ KL-3
Uterotrophic assay (Similar to OECD Guideline 440; GLP) – (anti)Estrogenic activity	Rats/ 100, 300 and 1000 mg/kg bw/day SC / 3 days	BP-1 showed both estrogenic and anti-estrogenic response	(Akahori et al., 2008)/ KL-2
Uterotrophic assay (Comparable to OECD Guideline 440; non-GLP) Estrogenic activity	Rats/ 20, 100 and 500 mg/kg bw/day intraperitoneal / 3 days	BP-1 showed slight increase in uterine weight in rats after intraperitoneal exposure, indicating weak estrogenic response	(Suzuki et al., 2005)/ KL-2
Estrogenic activity (non-guideline; non GLP)	Mice/ 200 mg/kg bw SC every 2 day for 8 weeks	BP-1 exerted estrogenic effects (similar to E2) by stimulating the proliferation of BG-1 ovarian cancer via the estrogen receptor signalling pathway associated with the cell cycle	(Park et al., 2013)/ KL-2/3
Level 4: *In vivo* assays providing data on ED adversity			
Sub-chronic toxicity study (non-guideline; non-GLP)	Rats / 0, 190, 600 and 1900 mg/kg bw/day dietary/ 90 days	BP-1 showed depressed growth rate, quantitative changes in erythrocyte and leucocyte numbers and lesions in liver and kidney higher	(Homrowski, 1968)/ KL-2

		doses. No ED adversity related information was given in this study. The systemic NOAEL was set at 190 mg/kg bw/day	

Level 5: *In vivo* assays providing more comprehensive data on adverse effects on ED related endpoints over more extensive parts of the life cycle of the organism

No information/studies available

<u>Assessment of the evidence</u>

Existing data and non-test information (OECD Conceptual Framework Level 1)

No existing data and no non-test information for BP-1 were identified from the literature.

***In vitro assays providing data about selected ED mechanisms / pathways* (OECD Conceptual Framework Level 2)**

***In vitro* high throughput screening (HTS) assays from the US EPA ToxCast program**

BP-1 has been tested as part of the ToxCast program of the US EPA, which currently contains 1057 ED-specific *in vitro* high throughput screening (HTS) assays, addressing the **E** (estrogen), **A** (androgen), **T** (thyroid) and **S** (steroidogenesis) modalities and are being used as part of the US EPA's Endocrine Disruptor Screening Program (EDSP21).

Under the EDS-21 program, BP-1 has been tested in 105 *in vitro* HTS assays (accessed in August 2021), including 42 (E=17; A=13; T=11; S=1) ED specific assays and 17 (E=4; A=4; T=8; S=1) paired cytotoxicity/specificity assays. Positive results are considered "active" and are reported as the concentration at which 50% of the maximum response is achieved (AC50, in µM). However, a positive result in a specific assay does not necessarily mean an adverse outcome. At times, this may be due to a burst of cellular responses indicative of cytotoxicity and not to a specific chemical-receptor interaction (Silva *et al.*, 2015). A careful review of the data to assess the reliability and relevance of the results is therefore required (ToxCast Owner's Manual, 2018).

As presented in [Applicant's Table 10], BP-1 was found to be active in 26 (E=16; A=7; T=2; S=1) out of 42 ED relevant assays (accessed in August 2021), out of which 5 have been flagged as potentially 'false positive8' by the automated analysis tool from the US EPA (ToxCast pipeline (tcpl) package9). Therefore, a closer review of the dose response curves was performed for each of the modalities. All results can be accessed from the CompTox dashboard10.

Estrogen receptor (ER) assays

BP-1 was tested in 17 ER-based HTS assays targeting the ER signalling pathway [Applicant's] Figure 1 in Annex IV], for the graphical representation of the testing points targeted by the *in vitro* assays for the ER pathway in ToxCast). The 17 assays including 13 agonistic and 4 antagonistic assays were conducted in different human, bovine, or mouse cell lines at concentrations ranging from 0.001 to 200 µM.

In the ER agonistic pathway, BP-1 was active in 6 out of 13 assays targeting receptor dimerization (node 1, N1; n=6), RNA transcription (N4; n=4), protein induction (N5; n=2) or ER-induced cell proliferation (N6; n=1) [Applicant's Table 10]. A review of the dose response

curves for all the active assays showed that BP-1 exceeded the cut-off threshold for a positive response, with AC50 values ranging from 1.16 to 28.4 µM. For perspective, the AC50 values were over 100 to 1,000,000 times higher than the AC50 value of the positive controls (17β-estradiol or 17alpha-Ethinylestradiol), indicating weak activity or low potency. Furthermore, BP-1 was found to be active in three out four antagonistic assays targeting receptor binding (R2; n=3) and RNA transcription (N10; n=1). One of the three active assay targeting receptor binding (NVS_NR_mERa) has been flagged as potentially 'false positive' by the automated analysis tool from the US EPA, which is also evident from the dose response curve to have than 50% efficacy (see Annex III). The AC50 values of the three active assays were determined to range from 1.33 to 9.02 µM, which is over 10,000-fold lesser compared the positive control (17β-estradiol), suggesting weak binding potential. Further, due to the small number of assays evaluating the antagonistic activity and the US EPA criterion for the interpretation of an active result (i.e., >5 active assays in a pathway) (Judson *et al.*, 2010), the anti-estrogenic activity of BP-1 could not be concluded based on the HTS assays.

In conclusion, considering the above information BP-1 is assessed to have only very weak estrogenic activity in the HTS assays. No conclusion could be reached for the anti-estrogenic activity.

Androgen receptor (AR) assays

BP-1 was tested in 13 AR-based HTS assays targeting the AR signalling pathway [Applicant's Figure 2 in Annex IV], for the graphical representation of the testing points targeted by the *in vitro* assays for the AR pathway in ToxCast). The 13 assays including 7 agonistic and 6 antagonistic assays were conducted in a hamster ovary, rat prostate and various human cell lines at concentrations ranging from 0.001 to 200 µM.

In the AR agonistic pathway, BP-1 was active in one out of seven assays targeting receptor binding (R1; n=1), co-factor recruitment (N2; n=2), RNA transcription (N4; n=3) or protein production (N5; n=1) (N6; n=1) [Applicant's Table 10]. However, this active assay targeting the cofactor recruitment (OT_AR_ARSRC1_0960) has been flagged as potentially 'false positive' by the automated analysis tool from the US EPA. This is confirmed by review of the dose response curve [Applcant's Annex III].

Further, in the AR antagonistic pathway, BP-1 was active in five out of six assays targeting receptor binding (R2; n=3 out of 4) and RNA transcription (N10; n=2). However, two of the five active assays (i.e., UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist and TOX21_AR_BLA_Antagonist_ratio) have been flagged as potentially 'false positive[11]' by the automated analysis tool from the US EPA, which is also evident from the dose response curve [Applcant's Annex III]. The AC50 of the active assays were determined to range from 12.7 to 39.1 µM, which is about 63 to 6,808 times higher than the AC50 values of the known reference substances (e.g., Nilutamide/Cyproterone acetate) [Applicant's Table 10].

In conclusion, considering the above information, BP-1 can be considered to show no true androgenic activity and only a weak anti-androgenic activity in the HTS assays.

Thyroid assays

BP-1 was tested in 11 thyroid HTS assays consisting of 5 agonistic and 6 antagonistic assays involving the thyroid hormone receptors and enzymes in rat and human cell lines, at concentrations ranging from 0.001 to 200 µM. Out of the 11 HTS assays, BP-1 was active in two assays: one assay testing the thyroid peroxidase enzyme inhibition (TPO) and the other targeting the RNA transcription in the thyroid hormone receptors in rat pituitary gland cell line [Applicant's Table 10]. However, the RNA transcription-based assay (TOX21_TR_LUC_GH3_Antagonist) was flagged as potentially 'false positive[12]' by the automated analysis tool from the US EPA, which is also evident from the dose response curve [Applicant's Annex 3]. Further, the TPO inhibition assay showed an AC50 value of 4.1 µM, which is about 70 times higher than the AC50 values of the positive control (methimazole). In addition, BP-1 was inactive in the remaining 9 assays involving the receptors of thyroid releasing hormone (TRH), thyroid stimulating hormone (TSH) or thyroid hormones (TR) and one assay measuring the inhibition of the mono-oxygenase enzyme levels [Applicant's Table

1 10].

2 In conclusion, considering the above information BP-3 is assessed to have a weak TPO
3 inhibiting activity in the HTS assays.

4 ***Steroidogenesis assays***

5 BP-1 was tested only in an aromatase (CYP19A1) inhibition assay, which is a luciferase-
6 reporter gene-based assay conducted using human breast cancer cell line, MCF-7. In this
7 assay, the cells were exposed to BP-1 at concentrations ranging from 0.001 to 90 μM for 24
8 hours. BP-3 was found to be inactive in this assay indicating no impact on the aromatization
9 of androgens to estrogens.

1 **Table 10:In vitro relevant ToxCast assays and results for BP-1**

Modality	ID	Assay	Effect type	Effect direction	Results	AC50 (µM)	Flag	Additional remarks
Estrogen	1	OT_ER_ERaERa_0480	Receptor dimerization (N1)	Agonist	ACTIVE	22.7	-	Potency: ca. 904 times less compared to the reference substance (17b-estradiol)
Estrogen	2	OT_ER_ERaERa_1440	Receptor dimerization (N1)	Agonist	ACTIVE	23.2	-	Potency: ca. 974 times less compared to the reference substance (17b-estradiol)
Estrogen	3	OT_ER_ERbERb_0480	Receptor dimerization (N1)	Agonist	ACTIVE	3.7	-	Potency: ca. 430 times less compared to the reference substance (17b-estradiol)
Estrogen	4	OT_ER_ERbERb_1440	Receptor dimerization (N1)	Agonist	ACTIVE	4.74	-	Potency: ca. 615 times less compared to the reference substance (17b-estradiol)
Estrogen	5	OT_ER_ERaERb_0480	Receptor dimerization (N1)	Agonist	ACTIVE	7.44	-	Potency: ca. 459 times less compared to the reference substance (17b-estradiol)
Estrogen	6	OT_ER_ERaERb_1440	Receptor dimerization (N1)	Agonist	ACTIVE	16.7	-	Potency: ca. 864 times less compared to the reference substance (17b-estradiol)
Estrogen	7	ATG_ERE_CIS_up	Receptor (trans) activation (N4)	Agonist	ACTIVE	1.49	-	Potency: ca. 166 times less compared to the reference substance (17b-estradiol)
Estrogen	8	ATG_ERa_TRANS_up	Receptor (trans) activation (N4)	Agonist	ACTIVE	1.16	-	Potency: ca. 116 times less compared to the reference substance (17alpha-Ethinylestradiol)
Estrogen	9	TOX21_ERa_BLA_Agonist_ratio	Receptor (trans) activation (N4)	Agonist	ACTIVE	28.4	-	Potency: ca. 217,245 times less compared to the reference substance (17b-estradiol)
Estrogen	10	TOX21_ERa_LUC_VM7_Agonist	Receptor (trans) activation (N4)	Agonist	ACTIVE	10.2	-	Potency: ca. 1,018,819 times less compared to the reference substance
Modality	**ID**	**Assay**	**Effect type**	**Effect direction**	**Results**	**AC50 (µM)**	**Flag**	**Additional remarks**

Estrogen	11	OT_ERa_EREGFP_0120	Protein induction/gene expression (N5)	Agonist	ACTIVE	6.29	-	Potency: ca. 200,279 times less compared to the reference substance (17b-estradiol)
Estrogen	12	OT_ERa_EREGFP_0480	Protein induction/gene expression (N5)	Agonist	ACTIVE	8.4	-	Potency: ca. 46,894 times less compared to the reference substance (17b-estradiol)
Estrogen	13	ACEA_ER_80hr	Cell proliferation (N6)	Agonist	ACTIVE	4.45	-	Potency: ca. 717 times less compared to the reference substance (17b-estradiol)
Estrogen	14	NVS_NR_bER	Receptor binding (R2)	Antagonist	ACTIVE	9.02	-	Potency: ca. 37,050 times less compared to the reference substance (17b-estradiol)
Estrogen	15	NVS_NR_hER	Receptor binding (R2)	Antagonist	ACTIVE	1.52	-	Potency: ca.50,802 times less compared to the reference substance (17b-estradiol)
Estrogen	16	NVS_NR_mERa	Receptor binding (R2)	Antagonist	ACTIVE	1.33	False positive - Less than 50% efficacy	
Estrogen	17	TOX21_ERa_BLA_Antagonist_ratio	Receptor (trans) activation (N10)	Antagonist	INACTIVE	-	-	
Estrogen	18	TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2	Specificity assay	-	INACTIVE	-	-	
Estrogen	19	TOX21_ERa_BLA_Antagonist_viability	Viability assay	-	INACTIVE	-	-	
Estrogen	20	TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2_viability	Viability assay	-	INACTIVE	-	-	
Estrogen	21	ACEA_ER_AUC_viability	Viability assay	-	INACTIVE	-	-	
Androgen	22	UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Agonist	Receptor binding (R1)	Agonist	INACTIVE	-	-	
Androgen	23	OT_AR_ARSRC1_0480	Cofactor Recruitment (N2)	Agonist	INACTIVE	58.4	False negative -	No dose response (none of the tested concentrations exceeded the cut-off of 20% activity)

							Borderline inactive	
Androgen	24	OT_AR_ARSRC1_0960	Cofactor Recruitment (N2)	Agonist	ACTIVE	60.1	False positive - Less than 50% efficacy: Only highest concentration above baseline, active	Only the highest concentration, 100 µM, above the baseline active or cut-off of 20%; AC50 exceeds cytotoxicity limit of 20.01 µM indicating non-specific activity; Potency: ca.181,438 times less compared to the reference substance (4,5-alpha-Dihydrotestosterone)
Androgen	25	ATG_AR_TRANS_up	Receptor (trans) activation (N4)	Agonist	INACTIVE	-	-	
Androgen	26	TOX21_AR_BLA_Agonist_ratio	Receptor (trans) activation (N4)	Agonist	INACTIVE	-	-	
Androgen	27	TOX21_AR_LUC_MDAKB2_Agonist	Receptor (trans) activation (N4)	Agonist	INACTIVE	-	-	
Androgen	28	OT_AR_ARELUC_AG_1440	Protein induction/gene expression (N5)	Agonist	INACTIVE	-	-	
Androgen	29	NVS_NR_cAR	Receptor binding (R2)	Antagonist	ACTIVE	12.7	-	Potency: ca.318 times less compared to the reference substance (Cyproterone acetate)
Androgen	30	NVS_NR_hAR	Receptor binding (R2)	Antagonist	ACTIVE	25.2	-	Potency: 4816 times compared to the reference substance (Nilutamide) and ca.28 times compared to the reference substance (Cyproterone acetate)
Androgen	31	NVS_NR_rAR	Receptor binding (R2)	Antagonist	INACTIVE	-	-	
Androgen	32	UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist	Receptor binding (R2)	Antagonist	ACTIVE	39.1	False positive - Only highest	Only the highest concentration, 100 µM, above the baseline active or cut-off of 37.98%; AC50

							concentration above baseline, active	exceeds cytotoxicity limit of 20.01 µM indicating non-specific activity; Potency: ca.6808 times less compared to the reference substance (Cyproterone acetate)
Androgen	33	TOX21_AR_BLA_Antagonist_ratio	Receptor (trans) activation (N10)	Antagonist	ACTIVE	35	False positive - Less than 50% efficacy	AC50 exceeds cytotoxicity limit of 20.01 µM indicating non-specific activity; Potency: ca.63 times less compared to the reference substance (Cyproterone acetate)
Androgen	34	TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881	Receptor (trans) activation (N10)	Antagonist	ACTIVE	26.6	-	Potency: ca. 66 times less compared to the reference substance (Nilutamide)
Androgen	35	TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881	Specificity assay	-	INACTIVE	-	-	
Androgen	36	TOX21_AR_BLA_Antagonist_viability	Viability assay	-	ACTIVE	113	False positive - Less than 50% efficacy: Only highest concentration above baseline, active	Viability assay
Androgen	37	TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881_viability	Viability assay	-	INACTIVE	-	-	
Androgen	38	TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881_viability	Viability assay	-	INACTIVE	-	-	
Thyroid	52	TOX21_TRA_COA_Agonist_Followup_ratio	Cofactor recruitment	Agonist	INACTIVE	-	-	
Thyroid	39	ATG_THRa1_TRANS_up	Receptor (trans) activation	Agonist	INACTIVE	-	False negative -	No dose response (none of the tested concentrations exceeded

							Multiple points above baseline, inactive	the cut-off of 1.12log2 fold change)
Thyroid	40	TOX21_TSHR_Agonist_ratio	Receptor (trans) activation	Agonist	INACTIVE	-		
Thyroid	41	TOX21_TR_LUC_GH3_Agonist	Receptor (trans) activation	Agonist	INACTIVE	6.91	False negative - Multiple points above baseline, inactive	No dose response (none of the tested concentrations exceeded the cut-off of 20% activity)
Thyroid	42	TOX21_TR_LUC_GH3_Agonist_Followup	Receptor (trans) activation	Agonist	INACTIVE	3.35	False negative - Multiple points above baseline, inactive: Borderline inactive	No dose response (none of the tested concentrations exceeded the cut-off of 20% activity)
Thyroid	43	TOX21_TSHR_Antagonist_ratio	Receptor (trans) activation	Antagonist	INACTIVE	-	-	
Thyroid	44	TOX21_TR_LUC_GH3_Antagonist	Receptor (trans) activation	Antagonist	ACTIVE	87	False positive - Less than 50% efficacy: Borderline active: Only highest concentration above baseline, active	Only the highest concentration, 100 µM, above the baseline active or cut-off of 22.43%; AC50 exceeds cytotoxicity limit of 20.01 µM indicating non-specific activity
Thyroid	45	ATG_THRa1_TRANS_dn	Receptor (trans) activation	Antagonist	INACTIVE	-	False negative - Multiple	No dose response (none of the tested concentrations exceeded

							points above baseline, inactive	the cut-off of 1.12log2 fold change)
Thyroid	46	NCCT_TPO_AUR_dn	Enzyme activity (regulation of catalytic activity)	Antagonist	ACTIVE	4.19	-	Potency: ca. 70 times less compared to the reference substance (methimazole (MMI))
Thyroid	47	NCCT_QuantiLum_inhib_2_dn	Enzyme activity (regulation of catalytic activity)	Antagonist	INACTIVE	-	-	
Thyroid	48	NVS_GPCR_rTRH	Receptor binding	Antagonist	INACTIVE	-	-	
Thyroid	49	TOX21_TR_LUC_GH3_Antagonist_viability	Viability assay	-	INACTIVE	-	-	
Thyroid	50	NCCT_HEK293T_CellTiterGLO	Viability assay	-	INACTIVE	-	-	
Thyroid	51	TOX21_TSHR_wt_ratio	Viability assay	-	INACTIVE	-	-	
Thyroid	53	TOX21_TRB_BLA_Agonist_Followup_ratio	Specificity assay	-	INACTIVE	-	-	
Thyroid	54	TOX21_TRB_BLA_Agonist_Followup_viability	Viability assay	-	INACTIVE	-	-	
Thyroid	55	TOX21_TRB_COA_Agonist_Followup_ratio	Specificity assay	-	INACTIVE	34.3	-	
Thyroid	56	TOX21_TR_RXR_BLA_Agonist_Followup_ratio	Specificity assay	-	INACTIVE	-	-	
Thyroid	57	TOX21_TR_RXR_BLA_Agonist_Followup_viability	Viability assay	-	INACTIVE	-	-	
Steroidogenesis	58	TOX21_Aromatase_Inhibition	Enzyme activity (regulation of transcription factor activity)	Antagonist	INACTIVE	-	-	

Steroidogenesis	59	TOX21_Aromatase_Inhibition_viability	Viability assay	-	ACTIVE	91.2	False positive - Less than 50% efficacy: Only highest concentration above baseline, active	Viability assay

1

In vitro endocrine mechanistic assays from literature
A number of well-conducted *in vitro* studies are available for BP-1. Studies are presented in the following sections according to the type of receptor binding assay.

Estrogen receptor (ER) assays
The estrogen receptor binding potential of BP-1 was tested using uterine cytosol (estrogen receptor) preparation from ovariectomized Sprague-Dawley rats. After adding BP-1 solution (10-5 - 1000 µM) and 1 nM of [3H] E2 to uterine cytosol preparation, the solution mixture was incubated for 20 hours at 4°C. Then radioactivity was measured using a liquid scintillation counter. Data for BP-1 and the E2 standard curve were plotted as percent [3H]-E2 bound versus molar concentration, and the IC50 (50% inhibition of [3H]-E2 binding) for the chemical determined. The relative binding affinity (RBA) for BP-1 was calculated by dividing the IC50 of E2 by the IC50 of the BP-1 and was expressed as a percent (E2 = 100). The IC50 value of the BP-1 was 36.5 µM, while E2 displayed an IC50 of approximately 8.99 x 10-4 µM. The receptor-binding affinity of BP-1 was 0.002 (% of EE). The study author concluded that BP-1 was a weak ER binder in the receptor-binding assay (Blair *et al.*, 2000).

An *in vitro* assay was conducted to study the role of metabolism on estrogenicity via estrogen receptor binding of BP-1 using Yeast Two-Hybrid Assay in the presence of S9-mix. To a tube containing the S-9 mix (rat liver supernatant fraction induced phenobarbital and 5,6-benzoflavone), 10 µL of BP-1 in DMSO was added and incubated at 37°C for 4 hours. After incubation with the S-9 mix, the mixture was stored at -8°C until their application to the yeast two-hybrid system (Yeast cells carrying the pGBT9-estrogen receptor ligand binding domain and pGAD424-TIF2 plasmids). The heat-inactivated S-9 extract was prepared by incubation at 95°C for 5 min and used for the negative control experiments. E2 was used as positive control. Maximum ß-galactosidase activity induced by incubation with E2 was obtained at concentrations of 1.0 X 10-8 M (0.01 µM) and higher. The REC10 values for E2 and BP-1 were reported to be 1.7 X 10-10 M (0.00017 µM) and 6.5 X 10-7 M (0.65 µM), respectively. Under the study conditions, the estrogenicity of BP-1 was found to be reduced by incubation with the active-S9 mix (Takatori *et al.*, 2003). Based on the REC10 values, BP-1 can be considered to be 1000 times less potent than E2.

The estrogen receptor binding potential of BP-1 was tested using recombinant human estrogen receptor ligand binding domain (hERα-LBD) fused with glutathione S-transferase and expressed in *E. coli*. After adding BP-1 solution (10-5 - 100 µM) and 5 nM of [2,4,6,7,16,17-3H] 17β-estradiol to a solution of recombinant hERa-LBD, the solution mixture was incubated for 1 hour at 25°C. Then radioactivity was measured using a liquid scintillation counter. The percent ratio (B/B0 (%)) of standard ligand ([3H] 17β-estradiol) bound to the receptor was calculated from the radioactivities of the solutions with and without the BP-1, subtracting the radioactivity due to non-specifically bound standard ligand to the receptor. The binding abilities of BP-1 to the receptor were evaluated by relative binding affinity (RBA), ratio of IC50 values to 17β-estradiol. The receptor-binding affinity of BP-1 was 0.0139 (% of EE). The study author concluded that BP-1 was a weak ER binder in receptor-binding assay (Yamasaki *et al.*, 2004).

The *in vitro* estrogenic activity of BP-1 was tested using recombinant human estrogen receptor ligand binding domain (hERα) EcoScreen cells derived from the Chinese hamster ovary cell line (CHO-K1). After adding 10-4 –10-10 M (i.e., 102 to 10-4 µM) BP-1 solution to a solution of recombinant hERα, the solution mixture was incubated for 16-24 hours at 25°C. DMSO as solvent control and 10-9 M (0.001 µM) E2 as positive control were used. Following the 24 hour culture, the luciferase substrate was added. After shaking for 5 mins at room temperature, the chemiluminescence was measured. The estrogenic activity of E2 was detectable at levels greater than 10-12 M (10-6 µM). The maximum induction was about 6-fold greater than the control at concentrations over 10-10 M (10-4 µM). BP-1 reported EC50 (half maximal effective concentration of test chemical) and PC50 (the concentration of a test chemical at which the response is 50% of the response induced by the positive control) values of 1.5×10-6 M (1.5 µM) and 7.7×10-7 M (0.77 µM), respectively. E2 reported EC50 and PC50 value of 1.5×10-11 M (1.5×10-5 µM). BP-1 showed approximately 1/100000 of the E2

activity. The study author concluded that BP-1 was a weak ER binder in receptor-binding assay (Kawamura *et al.*, 2005).

The *in vitro* (anti)estrogenic activity of BP-1 was evaluated in an ELISA-based estrogen receptor competitive binding assay (ER-ELISA) and a modified yeast two-hybrid estrogen assay, with and without addition of a rat liver preparation, S9 mix. In an ER-ELISA, BP-1 showed relative binding affinity to Diethylstilbestrol (DES, IC50 values of 8.3 and 12.9 nM for without and with S9 mix) values of 1.4 and 2.4 without and with S9 mix, respectively. In a yeast two-hybrid agonist assay, after 4 hour incubation with yeast cells 1 mM (103 µM) BP-1 showed relative activity (RA) to E2 (EC50 values of 1.24 and 20 nM for without and with S9 mix) values of 0.021 and 0.0052 without and with S9 mix. The results confirmed that BP-1 had weak agonistic effects in a two-hybrid assay without S9 mix. In a yeast two-hybrid antagonist assay, BP-1 reported EC50 values of >5000 nM (5 µM) without and with S9 mix, which confirmed that BP-1 had no antagonistic effects. Overall, it was concluded that BP-1 showed weak ER binding affinity but no ER antagonistic effect (Morohoshi *et al.*, 2005).

The estrogen receptor binding potential of BP-1 was tested using recombinant human estrogen receptor ligand binding domain (hERα-LBD) fused with glutathione S-transferase and expressed in *E. coli*. After adding BP-1 solution (10-5- 102 µM) and 0.5 nM of [2,4,6,7,16,17-3H] 17β-estradiol to a solution of recombinant hERa-LBD, the solution mixture was incubated for 1 hour at 25°C. Then radioactivity was measured using a liquid scintillation counter. The percent ratio (B/B0 (%)) of standard ligand ([3H] 17β-estradiol) bound to the receptor was calculated from the radioactivities of the solutions with and without the test substance, subtracting the radioactivity due to non-specifically bound standard ligand to the receptor. The binding abilities of test chemical to the receptor were evaluated by relative binding affinity (RBA), ratio of IC50 values to 17β-estradiol. The receptor-binding affinity of BP-1 was 0.0138 (% of EE). The study author concluded that BP-1 was negative in receptor-binding assay (Akahori *et al.*, 2008).

The *in vitro* estrogenic activity of BP-1 was studied in cell proliferation assay using MCF-7 human breast cancer cells expressing estrogen receptors. MCF-7 cells were incubated with 10–4–10–7 M (0.1 to 100 µM) BP-1 and 10–8–10–13 M (10–2–10–7 µM) E2 as positive control (10–8–10–13 M). An EC50 value for E2 was 1.22 pM (1.2×10-6 µM). BP-1 reported an EC50 of 2.08 µM, indicating potential estrogenic activity (Schlumpf and Lichtensteiger, 2001; Schlumpf *et al.*, 2004). Based on the EC50 values, BP-1'sestrogenic activity was 6 times less potent than E2.

The *in vitro* estrogenic activity of BP-1 was examined in recombinant yeast transactivation assay expressing rainbow trout estrogen receptor (rtERα). The results were compared to the ones obtained by using an assay with yeast expressing the human estrogen alpha (hERα), for receptor specificity. BP-1 had maximal responses of 114% compared to E2 in the rtERα assay and IC50 value of 79.9 µM (87 times less potent than E2). BP-1 showed a maximal response of 96% in the hERα assay with an IC50 of 1.15 µM (5000 times less potent than E2). The activity of BP-1 was relatively higher with rtERα than with hERα. The assay was valid, as the positive control E2 showed estrogenic activity in both the assays with an EC50 of 0.0181 and 2.9x10-4 µM respectively. Therefore, BP-1 can be concluded to have a strong estrogenic activity in both rtERα and hERα transactivation assays (Kunz *et al.*, 2006). BP-1 reported 87 times less potent activity than E2 in rtERα assay and 5000 times less potent activity than E2 in hERα assay.

The *in vitro* (anti)estrogenic activity of BP-1 was evaluated in a recombinant yeast transactivation assay expressing human estrogen receptor alpha (hERα) sub-type receptors. The agonistic assay was performed with 10 µM of BP-1 and the antagonistic activity was determined by co-incubation of BP-1 with the respective agonists, β-Estradiol (E2). E2 and 4-hydroxytamoxifen (4-HT) were used as the respective positive controls. Under the test conditions, BP-1 showed strong estrogenic activity (EC50: 1.15 µM with 96% efficacy). This effect concentration was 5,000 times less potent compared to the EC50 value of the E2

determined at 2.59x10-4 µM. Under the test conditions, no antiestrogenic effects were observed (Kunz and Fent, 2006).

An *in vitro* reporter gene assay was conducted to determine estrogenic activity of BP-1 using zebrafish liver cell line (ZFL) transfected an ERE-luciferase construct, yielding a ZELH (ZFL-ERE Luciferase Hygromycine) clone. ZELH-zfERα, ZELH-zfERβ1, ZELH and ZELH-zfERβ2 cell lines were exposed to BP-1 for 72 hours at 28°C. Luciferase activity was then determined in living cells. Relative estrogenic potencies (REPs) were determined as the ratio of EC50 of E2 to that of test chemical. E2 had higher potency on zfERβ1 and zfERβ2 than on zfERα, with EC50 of 0.027 nM (2.7 x 10-5 µM), 0.051 nM (5.1 x 10-5 µM), and 0.20 nM (20 x 10-5 µM), respectively. BP-1 EC50 and REP values in zfERβ2 and zfERα were 3859 nM (3.859 µM) and 1.3 x 10-5, 2195 nM (2.195 µM) and 9.3 x 10-5, respectively (5-6 fold less potent than E2). BP-1 was reported to have slightly higher affinity for the zfERα than for the beta subtypes. Under the *in vitro* test conditions, BP-1 was concluded to have very weak estrogenic activity (Cosnefroy *et al.*, 2012).

The *in vitro* estrogenic activity of BP-1 was examined in an estrogen response element (ERE)-luciferase reporter assay using human MCF-7 cells. In the assay, the cells were incubated with 0.001-500 µM BP-1 for 5 days. Cell numbers were increased in a concentration dependent manner from 0.01 µM to 1 µM. BP-1 was cytotoxic to MCF-7 cells at concentrations above 10 µM, so its apparent activities decreased above this concentration. In a competitive binding assay for estrogen receptor α (ERα), the affinity of 5 × 10-10 M to 5 × 10-4 M (5 × 10-4 µM to 500 µM) BP-1 to estrogen receptor was studied using the fluorescein-labelled 17β-estradiol. BP-1 displaced in a competitive manner the 17β-estradiol bound to its receptor. IC50 value of the BP-1 was 5 × 10-5 M (50 µM) (Nakagawa *et al*., 2002 [reference is of 2000]). Result for 17β-estradiol was not reported. Overall, BP-1 was considered to have weak estrogenic activity under the *in vitro* test conditions (Nakagawa and Suzuki, 2002).

The *in vitro* estrogenic activity of BP-1 was examined in an estrogen response element (ERE)-luciferase reporter assay using human MCF-7 cells. In the assay, the cells were incubated with 0.01-100 µM BP-1. BP-1 was cytotoxic to MCF-7 cells at concentrations above 10 µM, so its apparent activities decreased above this concentration. BP-1 reported an EC50 of 1.26 µM, indicating potential estrogenic activity (Suzuki *et al.*, 2005). Result for control groups were not reported.

A study was conducted to investigate the binding and activation of estrogen related receptor γ (ERRγ) as an alternative mechanism of BP-1 endocrine disruption toxicity. In a competitive fluorescence binding assay, ERRγ LBD was treated with BP-1 (102-106 nM) (0.1 to 103 µM) for 10 mins. It exhibited relatively weak binding affinity with ERRγ-LBD, with the IC50 127.9 µM and relative binding affinity (RBA) value of 5.6. The transcriptional activity of the BP-1 on ERRγ transfected HepG2 cells using ERRγ-mediated luciferase reporter gene assay was investigated with 102-106 nM (0.1 to 103 µM) concentration exposure for 48 hours. GSK4716, a known agonist, effectively enhanced ERRγ-mediated luciferase transcriptional activity, approximately by 2.4 folds at 10 µM, while antagonist 4-OHT inhibited transcriptional activity at a rate of 56%, indicating the validity of this assay. BP-1 showed enhancement with the lowest observed effective concentration (LOEC) of 3.12 µM. The study author concluded that BP-1 interacted with ERRγ directly and exerted agonistic activity towards ERRγ-mediated transcriptional pathway. Based on the reported BP-1 concentration in human blood and its LOEC on ERRγ obtained in this study, the study authors calculated hazard quotient (HQ) of BP-1 for the general population was <0.013, which indicated relatively low risk to the population at the current exposure levels. Overall, it can be concluded that BP-1 exhibited weak binding affinity with ERRγ-LBD (5.6 times less potent) and activated the transcriptional activity through ERRγ mediated pathway (Zheng *et al.*, 2020).

The *in vitro* estrogenic activity of BP-1 was investigated using human MCF-7 cell proliferation assay and CHOOSER assay (Chinese hamster ovary cell transformed with the gene encoding the human estrogen receptor (ER α) and an estrogen responsive promoter linked to a reporter gene). BP-1 induced dose dependent statistically significant cell proliferation compared with the vehicle control. When BP-1 was added to the cells along with ICI 182780, an estrogen receptor (ER) antagonist, the cell growth was reduced according to its doses. Therefore, the

cell proliferation was suggested to generate through ER. BP-1 was positive in CHOOSER assay, a new method of testing estrogenic activity of xenoestrogen. The relative activity (RA) value for E2 was set at 106. REC10, RA values for MCF-7 assay and CHOOSER assay were 1.2 µM, 4.2 and 15 µM, 35, respectively. BP-1 was confirmed to bind to ERα and ERβ using a human ER competitive binding assay against 17β-estradiol. IC50, relative binding affinity (RBA) values for ERα and Erβ were 86 µM, 44 and 2.2 µM, 8.6, respectively (Mutsumoto *et al*., 2005). Therefore, BP-1 can be concluded to have an estrogenic activity in both MCF-7 cell proliferation (4.2 times less potent than E2) and CHOOSER (35 times less potent than E2) assay.

The *in vitro* estrogenic activity of 0.01–10 µM BP-1 was evaluated in a battery of *in vitro* tests using human and fish ER from human and fish origin, i.e., reporter gene based assays, competitive binding, vitellogenin (Vtg) induction in isolated rainbow trout hepatocytes, and proliferation based assays. The estrogenic activity was measured in reporter gene-based assays using MCF-7-ERE-Luciferase-Neo (MELN) and HeLa-ERE-Luciferase-Neo (HELN) cell lines transfected with hERα only and hERα and hERβ respectively. In these assays, BP-1 was found to induce luciferase expression with an EC50 of 9.19 (MELN), 8.51 (HELN-ERα), 3.97 (HELN-Erβ) µM, compared to 1.4x10-5 (MELN), 1.9x10-5 (HELN-ERα), 6.7x10-5 (HELN-Erβ) µM for the positive control estrogen (E2) respectively, indicating a very weak activity (>106-fold). BP-1 induced 60% of maximal luciferase activity at 10 µM in HELN parental cell line devoid of ER. BP-1 displayed a preference for transactivation of hERβ rather than hERα. The binding affinity of BP-1 for hERβ were consistent with the estrogenic activities defined in the reporter gene assay system. Further, BP-1 was showing similar weak activity in the HELN transfected rainbow trout ERα-based assay with EC50 value of 18.43 µM. In whole cell competitive binding assays, BP-1 inhibited the binding of [3H]-E2 toward hERα, hERβ, and rtERα receptors in a concentration-dependent and competitive manner. The IC50 values for BP-1 were 3.190, 1.590 and 2.45 µM, whereas IC50 values for E2 were 0.12, 0.21 and 1.63 nM for these receptors (2-3 fold less potent). BP-1 inhibited proliferation in a clear dose-dependent manner when applied to HELN Erα and -ERβ cells. IC50 values in HELN-ERα and HELN-ERβ cells were 19 and 7553 nM, respectively. E2 results were not reported. BP-1 induced significant Vtg production in primary cultures of rainbow trout hepatocytes (PRTH) and reaching a maximal induction at 30 µM, which is an effect mediated by rtERα (Molina-Molina *et al.*, 2008). Under the *in vitro* test conditions, BP-1 was concluded to have very weak estrogenic activity (>106 times less potent than E2) and antiestrogenic activity (2-3 times less potent than E2).

The *in vitro* estrogenic activity of BP-1 via cell proliferation assay using BG-1 human ovarian cancer cells expressing estrogen receptors, when compared to E2, was evaluated. In the *in vitro* cell viability assay, BP-1 (0.01 to 10 µM) caused a statistically significantly increas in BG-1 cell growth, as did E2. The mechanism underlying BG-1 cell proliferation induced by BP-1 was shown to be related to the up-regulation of cyclin D1, a cell cycle progressor. Both BP-1 and E2 induced cell growth and up-regulation of cyclin D1 were reversed by co-treatment with an ER antagonist, suggesting that BP-1 may, similar to E2, mediate the cancer cell proliferation via an estrogen receptor-dependent pathway. However, the expression of p21 (regulator of cell cycle progression at G1 phase) was not altered by BP-1, though it was down-regulated by E2 (Park *et al.*, 2013).

The *in vitro* estrogenic activity of BP-1 on the proliferation and metastasis of MCF-7 human breast cancer cells expressing estrogen receptors were studied. The study of alterations in transcriptional and translational levels of proliferation and metastasis-related markers (cyclin D1, p21, and cathepsin D) was also performed. Treatment of the cells with BP-1 (0.01 to 10 µM) promoted the proliferation of MCF-7 cells in a manner that was similar to the positive control (E2). The addition of BP-1 also markedly induced the migration of MCF-7 cells in a manner that was similar to E2. Also, the BP-1 treatment with cells led to an increase in the expression of cyclin D1 and cathepsin D, and a decrease in p21 (at both transcriptional and translational levels). On treatment of the cells with BP-1 (10 µM) in the presence of an ER antagonist, ICI 182,780 (0.01 µM), the BP-1 induced growth of MCF-7 cells was restored to level of a control, indicating that BP-1 may promote proliferation of breast cancer cells through an ERα-dependent pathway. The study authors concluded that BP-1 may accelerate the

growth of MCF-7 breast cancer cells by regulating cell cycle-related genes and promote cancer metastasis through amplification of cathepsin D. Also, Benzopheneone-1 can be concluded to have estrogenic activity in MCF-7 cell proliferation assay (In Sol-Ji *et al.*, 2015).

The *in vitro* estrogenic activity of BP-1 was investigated using MCF-7 cell proliferation assay. The migration and invasion of MCF-7 were investigated. The MCF-7 cells possess intrinsic low motility, but increased motility was observed after exposure to the UV filters using three independent assay systems (scratch assay, live cell imaging and xCELLigence technology). The long-term exposure to the UV filters BP-1 at 10-5M following ≥20 weeks exposure showed increase in the migratory and invasive properties of both oestrogen responsive (MCF-7) and estrogen unresponsive (MDA-MB-231) human breast cancer cells, implying that their ability to increase cell motility was not confined to estrogen-responsive cells. Reduction in E-cadherin was observed following 24 weeks' exposure to 10−5 M (0.1 µM) of BP-1 (Alamer and Darbre, 2018). Therefore, BP-1 can be concluded to have estrogenic activity in MCF-7 cell proliferation assays.

Androgen receptor based in vitro assays

The *in vitro* (anti)androgenic activity of BP-1 was tested using recombinant human androgen receptor ligand binding domain (hAR) EcoScreen cells derived from the Chinese hamster ovary cell line (CHO-K1). After adding 10-4 –10-10 M (i.e., 102 to 10-4 µM) BP-1 solution to a solution of recombinant hAR, the solution mixture was incubated for 16-24 hours at 25°C. For hAR medicated reporter gene antagonist assay, 5×10-10 M (5×10-4 µM) of DHT was used for the final sample diluent. Following the 24 hour culture, the luciferase substrate was added. After shaking for 5 mins at room temperature, the chemiluminescence was measured. DMSO as solvent control, 0.01 µM DHT as positive control and 1 ug/mL cycloheximide as cell toxicity positive control were used. The androgenic activity was not detectable at <100 µM. BP-1 reported EC50 and PC50 values of >1×10-4 M (>100 µM) and 1.8×10-5 M (18 µM), respectively. DHT reported EC50 value of 1.6×10-10 M (1.6×10-4 µM); whereas hydroxy flutamide reported IC50 value of 1×10-7 M (0.1 µM). The study author concluded that BP-1 had no or weak androgenic activity and weak antiandrogenic activity (Kawamura *et al.*, 2005). BP-1 was 100 times less potent than hydroxy flutamide.

The *in vitro* (anti)androgenic activity of BP-1 was evaluated in a recombinant yeast transactivation assay expressing human androgen receptor (hAR). The agonistic assay was performed with 10 µM of BP-1 and the antagonistic activity was determined by co-incubation of BP-1 with the standard agonist, DHT. DHT and flutamide were used as the respective positive controls. Under the test conditions, no androgenic effects were observed. However, BP-1 showed anti-androgenic activities with IC50 value at 0.69 µM which was about 4 times more potent compared to the flutamide with an IC50 value of 4.32 µM. Therefore, BP-1 was concluded to have potent anti-androgenic activity under *in vitro* test conditions (Kunz and Fent, 2006).

The (anti)androgenic activity of BP-1 was evaluated in an androgen response element (ARE)-based reporter gene assay using rat fibroblast (NIH3T3) cell line. The cells were incubated with BP-1 at concentrations ranging from 10-5 to 10-8 M (0.01 to 10 µM) for 24 hours. No androgenic activity was reported. When BP-1 was added to the DHT assay system in the concentration range of 0.01 to 10 µM, the AR activity of 0.0001 µM DHT was inhibited concentration-dependently, with an IC50 value of 10 µM. No control group values were reported. The study author concluded that BP-1 had no androgenic activity and weak antiandrogenic activity (Suzuki *et al.*, 2005).

The *in vitro* and *ex vivo* (anti)androgenic activities of BP-1 were evaluated. HEK-293 cells transiently expressing human 17β-hydroxysteroid dehydrogenase type 3 (HSD3) were incubated with 20 µM of the BP-1 and 200 µM androgen (AD) for 45 min, followed by determination of testosterone formation. BP-1 concentration-dependently inhibited 17β-HSD3 (IC50 1.05 µM). To assess whether BP-1 inhibits testosterone synthesis in an endogenous system, *ex vivo* assays with mouse and rat testis were performed. Freshly isolated tissue from decapsulated testes was incubated with 1 µM radiolabelled AD for 15 min in the presence or absence of BP-1. Inhibition of testosterone formation was observed in testes isolated from either mice or rats. Conversion of AD to testosterone in mouse testis tissue was inhibited by

more than 80% at BP-1 concentrations of 5 μM and higher. Similar observations were made in assays with rat testis tissue. To evaluate the relevance of the observed anti-androgenic activities of the BP-1 inhibiting 17β-HSD3-dependent testosterone synthesis, its direct effects on AR in reporter-gene transactivation assays was determined using HEK-293 cells. Cells were incubated with 0.2 nM testosterone and either vehicle (0.1% DMSO) or BP-1 for 24 hours, followed by determination of the ratio of β-galactosidase and luciferase activities. BP-1 did not activate the AR at concentrations up to 20 μM but inhibited testosterone-dependent AR activation with IC50 value of 5.7 μM (about 5 times lower than that for the AR). Overall, the study authors concluded that BP-1 inhibits 17β-HSD3 dependent testosterone formation in intact cells as well as *ex vivo* in freshly isolated mouse and rat testes. BP-1 also directly antagonized testosterone-dependent AR activation (about 5 times lower than that for the AR) (Nashev *et al.*, 2010).

A study was performed to evaluate the effects of BP-1 on prostate cancer progression including cell proliferation and migration using LNCaP PCa cells. To evaluate effect of BP-1 (0.01 to 10 μM for 4 days) on the proliferation of LNCaP cells, MTT assay was performed. In addition, to demonstrate the connection between BP-1 and AR signalling pathway, LNCaP cells were co-treated with BP-1 (1 μM) along with bicalutamide, atypical AR antagonist (0.001 μM) for 6 days and the cell viability was measured by MTT assay. BP-1 increased the viability of LNCaP prostate cancer cells at concentrations of 1 μM and 0.1 μM. In the MTT assay, when the cells were co-treated with BP-1 (1 μM) and bicalutamide (0.001 μM), the cell viability that was increased by Benzopheonone-1 alone was statistically significantly reduced, showing that the proliferative effects of BP-1 on LNCaP cells was mediated by the androgen receptor signalling pathway. The treatment of BP-1 (1 μM) increased the cell migration compared to solvent control. In parallel with changes in cell viability levels, the migration activity of LNCaP cells increased by BP-1 was significantly reduced by a co-treatment with an AR antagonist (0.001 μM biclutamide), showing that the stimulation effects on LNCaP cell migration of BP-1 were mediated via AR signalling pathway. These results suggest that the proliferative and migration effects of BP-1 on LNCaP cells was mediated by the androgen receptor signalling pathway (CIR, 2021; Kim *et al.*, 2015).

Thyroid receptor based in vitro assays

A study was performed to evaluate the thyroid-disrupting potential of BP-1 using rat pituitary (GH3) and thyroid follicle (FRTL-5) cell lines. BP-1 at 0, 1, 3.2, 10, 32 μM (0.2-6.9 mg/L) concentrations was incubated with GH3 cell line and at 0, 10, 32, 100, and 320 μM (2.1-68.6 mg/L) concentrations was incubated with FRTL-5 cell line. For GH3, T3 was used as a positive control at 1 nM; whereas for FRTL-5, TSH (10 mU/mL) was used as a positive control. DMSO was vehicle control. In GH3 cells, three genes involved in central regulation of the thyroid system were observed, which include Thyrotropin-releasing hormone receptor (Trhr), Thyroid stimulating hormone beta (Tshβ), and Thyroid hormone receptor beta (Trβ). In FRTL-5 cells, four genes responsible for thyroid hormone synthesis were analyzed in the cells, which include Thyroid-stimulating hormone receptor (Tshr), Sodium/iodide symporter (Nis), Thyroglobulin (Tg), and Thyroid peroxidase (Tpo). Significant downregulation of the Tshβ, Trhr, and Trβ genes was observed following exposure to BP-1, even at doses of 10 μM and below. This down-regulating pattern of the Tshβ, Trhr, and Trβ genes was similar to those observed following T3 exposure (1 nM). The Nis gene was significantly up-regulated following exposure to BP-1. Although statistical significance was not reached, the change in Tg gene transcription was relatively high, i.e., > 3.0-fold change after exposure to BP-1. In addition, exposure to BP-1 significantly down-regulated the Tpo gene. BP-1 did not affect the transcription level of the Tshr gene. Exposure to TSH significantly up-regulated Nis by up to 9.6-fold and at the same time, significantly down-regulated the Tshr, Tg, and Tpo genes. The results of this study indicate that BP-1 can alter thyroid hormone balances by influencing the central regulation and metabolism of hormones (Lee *et al.*, 2018).

Steroidogenesis based in vitro assays

An *in vitro* assay was conducted to study the effect of BP-1 exposure on steroids using H295R cells. H295R cells were incubated for 48 hours with vehicle (0.1 % DMSO) or 1-10 μM BP-1. Changes in steroid levels were measured by LC-MS. Data are expressed as a fold change

relative to the solvent control and represent the mean from the experiment, performed in triplicate (n=3). Steroid metabolites down regulated or up regulated by 1.5-fold or more were recorded to indicate trend changes. BP-1 did not produce 1.5-fold changes in steroids progestins (pregnenolone, 17α-hydroxypregnenolone, progesterone, 17α-hydroxyprogesterone), adrenal androgens (androstenedione, dehydroepiandrosterone, testosterone, 11β-hydroxy-androstenedione), mineralocorticoids (aldosterone, 11-deoxycorticosterone, corticosterone) and glucocorticoids (cortisol, 11-deoxycortisol) (Strajhar *et al.*, 2017). Overall, it can be considered that BP-1 did not have effect on steroidogenesis.

Overall evaluation of Level 2 studies

According to the Applicant:

BP-1 showed activity in 26 out of 42 HTS assays addressing the E, A, T and S modalities. However, a closer review of the dose response curves of the positive assays indicates weak estrogenic, weak anti-androgenic and weak TPO inhibiting activity, with minimum 100-fold, 60-fold and 70-fold lower potency compared to their respective reference standards. Further, BP-1 was not found to be active in the aromatase inhibition assay.

Based on the *in vitro* assays reported in the literature, BP-1 was found to have weak estrogenic activity which was >5 to 5000-fold less potent as compared to standard agonists. The EC50 of the ER agonistic assays were determined to range from 1.15 to 86 µM. Similarly, BP-1 did not show clear evidence for binding to rat AR and showed weak anti-androgenic activity via transcriptional activation (IC50 ranging from 0.69 to >100 µM), which is about 4 to >100 times less potent than the known antagonist reference substances (e.g., hydroxyflutamide). Furthermore, BP-1 was found to alter three genes involved in central regulation of the thyroid system which was >10,000 fold less potent as compared to standard agonist T3. In an *in vitro* steroidogenesis assay, BP-1 did not cause up or down regulation of steroid hormones which confirmed that it does not have any effect on steroidogenesis [Applicant's section 3.4.3.2]**.**

***In vivo* assays providing data about selected ED mechanisms / pathways (OECD Conceptual Framework Level 3)**

An uterotrophic assay was conducted with BP-1 in ovariectomised C57BL/6J adult female mice (age 8 weeks) according to OECD TG 440. The test substance in corn oil was administered orally and subcutaneously (SC) to 6 animals per dose group (0, 30, 100, 300 and 1000 mg/kg bw/day) for 7 consecutive days. For the agonistic activity detection, control animals received the vehicle (corn oil) and positive control animals received 17 α- ethynyl oestradiol (EE) at 6 µg/kg bw/day and 0.2 µg/kg/day by oral and SC routes, respectively. For antagonistic activity detection, both oral and SC studies of BP-1 dose groups and the vehicle dose groups were administered at reference doses of 0.6 µg/kg bw/day EE via SC route following 15 minutes exposure. BP-1 showed no antagonist effect by the oral route of exposure. By the SC route, clear agonistic and antagonistic effects were detected in a dose-dependent manner with statistical significance at higher doses. AG10 (interpolated dose that corresponded to 10% of the maximal agonistic uterotrophic effect) and AN50 (interpolated dose that suppresses the uterotrophic effect of the reference EE to 50% of the maximal uterotrophic response) values for BP-1 were reported to be 409.9 and 243.2 mg/kg/day, respectively. For AG10 calculation, the uterotrophic response of positive control EE Group was considered as 20% of the maximal increase above the concurrent vehicle control. For AN50 calculation, the reference EE Group was considered as 70% of the maximal uterotrophic response above concurrent vehicle control. Vehicle control and positive control results confirmed the study validity. The study author reported LOAEL values were 1000 mg/kg bw/day (highest tested dose) for oral agonist activity and 300 mg/kg bw/day for SC agonist and antagonist activity (Ohta *et al.*, 2012). The NOAEL was established at 300 mg/kg bw/day for oral agonist activity and 100 mg/kg bw/day for SC agonist and antagonist activity.

An uterotrophic assay was conducted with BP-1 in ovariectomised Crj:CD (SD) adult female rats (age 10-11 weeks). Test substance in olive oil was administered subcutaneously to animals (0, 100, 250 and 625 mg/kg bw/day) for 3 consecutive days. For the agonistic activity detection, control animals received the vehicle (olive oil) and positive control animals received

EE at 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 µg/kg bw/day by SC route. BP-1 produced a significant effect on both wet and blotted uterine weights at 625 mg/kg bw/day. Based on these results, the study author calculated the best-fit dose–response curves for EE and BP-1 using the Hill equation and obtained ED10s and ED50s for their uterotrophic effects. ED10 and ED50 values of EE were 1.4 × 10-4 (wet uterine weight), 5.3 × 10-5 (blotted uterine weight) and 3.7 × 10-4 (wet uterine weight), 2 × 10-4 (blotted uterine weight) mg/kg bw/day, respectively. ED10 and ED50 values of BP-1 were 590 (log wet/log body weight, mg/kg bw/day), 540 (log blotted/log body weight, mg/kg bw/day) and 650 (log wet/log body weight, mg/kg bw/day), 630 (log blotted/log body weight, mg/kg bw/day) mg/kg bw/day, respectively. The relative estrogenic potency of BP-1 against EE was estimated to be about 1/10,000,000 when calculated using the ED10 for blotted weight. The LOAEL in the study was reported to be 625 mg/kg bw/day (Koda *et al.*, 2005). The NOAEL was established at next lower dose of 250 mg/kg bw/day.

An uterotrophic assay was conducted with BP-1 in immature female Crj:CD (SD) rats (20 days old), in compliance with GLP. BP-1 was administered subcutaneously to 6 animals per dose group at 100, 300 and 1000 mg/kg bw/day for 3 consecutive days. Ethynyl estradiol (EE) in olive oil was also subcutaneously injected into the back of some rats in a dose of 0.6 µg/kg bw/day on three consecutive days after administration of the chemical at the same doses. A vehicle control group was injected with olive oil alone, and a positive control group was injected with EE after administration of olive oil. A group injected with the estrogen-antagonist chemical tamoxifen in a dose of 1 mg/kg bw/day plus EE was also established to confirm the reliability of this study. No clinical abnormalities were observed in any of the groups, and body weight increased normally in all groups. The uterine weight of the rats given EE was higher than that of the rats given vehicle alone, and the uterine weight of the rats given tamoxifen plus EE was lower than that of the rats given EE, confirming the reliability of this study. Uterine weight was significantly higher in all BP-1 treatment groups and was significantly lower for BP-1 plus EE groups at 300 and 1000 mg/kg bw/day (Yamasaki *et al.*, 2004). The LOAEL in the study can be considered to be 100 mg/kg bw/day.

An uterotrophic assay was conducted using BP-1 in immature female Long Evans rats. BP-1 was administered orally to animals at 2-1200 mg/kg bw/day for 3 consecutive days. Control animals received the vehicle (olive oil) and positive control animals received 17 β-oestradiol. BP-1 administration did not show overt toxicity. The uterine weight in the groups dosed with BP-1 was increased compared with the vehicle group. Therefore, under the conditions of the study, BP-1 showed uterotrophic (estrogenic) effect in rats when compared with the control group (Schlumpf *et al.*, 2004). No more details about NOAEL/LOAEL were reported in the study.

An uterotrophic assay was conducted using BP-1 in immature female Crj:CD (SD) rats (20 days), in compliance with GLP. BP-1 was administered subcutaneously to 6 animals per dose group at 100, 300 and 1000 mg/kg bw/day for 3 consecutive days. Ethynyl estradiol (EE) in olive oil was also subcutaneously injected into the back of some rats in a dose of 0.6 µg/kg bw/day on three consecutive days after administration of the chemical at the same doses. A vehicle control group was injected with olive oil alone, and a positive control group was injected with EE after administration of olive oil. A group injected with the estrogen-antagonist chemical tamoxifen in a dose of 1 mg/kg bw/day plus EE was also established to confirm the reliability of this study. No clinical abnormalities were observed in any of the groups, and body weight increased normally in all groups. The uterine weight of the rats given EE was higher than that of the rats given vehicle alone, and the uterine weight of the rats given tamoxifen plus EE was lower than that of the rats given EE, confirming the reliability of this study. The log lowest effective doses (logLED, µmol/kg/day) for estrogenic and anti-estrogenic effects were 2.67 and 3.15 µmol/kg/day (equivalent to 0.553 and 0.652 mg/kg bw/day, after dividing by 4.83 conversion factor), respectively (Akahori *et al.*, 2008).

An uterotrophic assay was conducted using BP-1 in ovariectomised female F344 rats (9 weeks). BP-1 was administered intraperitoneally (IP) to 5 animals per dose group at 20, 100 and 500 mg/kg bw/day for 3 consecutive days. Control animals received the vehicle (corn oil) and positive control animals received 17 β-oestradiol at 50 µg/kg bw/day. BP-1 administration

1 did not decrease the body weight compared with other groups. The uterine weight in the
2 groups dosed with BP-1 was weakly increased compared with the vehicle group; however, it
3 was statistically significant at highest dose only. Therefore, under the conditions of the study,
4 BP-1 showed weakly uterotrophic (estrogenic) effect in rats when compared with the control
5 group (Suzuki *et al.*, 2005). The NOAEL in the study can be considered to be 100 mg/kg
6 bw/day.

7 A study was conducted to evaluate the ability of BP-1 to stimulate tumor growth via the
8 estrogen receptor signalling pathway in xenograft mice transplanted with BG-1 ovarian cancer
9 cells. 6 mice were injected SC with E2 (0.02 mg/kg) every 2 days for 8 weeks, and another
10 group of 6 mice was dosed SC with BP-1 (200 mg/kg bw). The vehicle control group was
11 dosed with corn oil. BP-1 or E2 treatment statistically significantly increased the tumour mass
12 formation (compared to corn oil vehicle) within 8 weeks. At histopathological examination,
13 the tumour sections of the E2 or BP-1 group displayed extensive cell formations with high
14 density and disordered arrangement. These results were supported by the increased number
15 of BrdUrd positive nuclei and the over-expression of cyclin D1 protein. The study authors
16 concluded that BP-1 exerts estrogenic effects (similar to E2) by stimulating the proliferation
17 of BG-1 ovarian cancer via the estrogen receptor signalling pathway associated with the cell
18 cycle (Park *et al.*, 2013).

19 **Overall evaluation of Level 3 studies**

20 The Level 3 *in vivo* mechanistic toxicity of BP-1 has been assessed on the basis of six
21 uterotrophic assays in rodents. All assays showed a weak uterotrophic (estrogenic) effect in
22 rats. The oral NOAEL for estrogenic effects was reported to be 300 mg/kg bw/day; whereas
23 the NOAELs were in the range of 100 to 250 mg/kg bw/day via the SC route. The IP NOAEL
24 was reported to be 100 mg/kg bw/day for estrogenic effects. In one uterotrophic assay in
25 rats, the log lowest effective doses (logLED, μmol/kg/day) for estrogenic or anti-estrogenic
26 effects were 2.67 and 3.15 (equivalent to 0.553 and 0.652 mg/kg bw/day), respectively.

27 No literature for BP-1 could be identified to assess its potential (anti)androgenic activity.

28

29 ***In vivo* assays providing data on adverse effects on ED related endpoints (OECD**
30 **Conceptual Framework Level 4)**

31 Except for the sub-chronic toxicity study with BP-1 for which only limited information on
32 methodologies and findings is available, no Level 4 studies could be identified for BP-1.
33 Therefore, data available on the analogue BP-3 were used for assessing this endpoint. The
34 studies are described below, as well as in [Applicant's] Sections 3.3.5 and 3.3.6**.**

35 ***Repeated dose toxicity and* carcinogenicity *studies***

36 No reproductive specific toxicity was reported in the limited detailed oral sub-chronic toxicity
37 study available with BP-1 in rats. The NOAEL was established at 190 mg/kg bw/day.

38 Further, sub-chronic oral toxicity studies with the analogue BP-3 in rodents, showed some
39 reproductive toxicity at high doses, which were always accompanied with overt systemic
40 toxicity. Reduction of sperm density and/or abnormal sperms was observed in rats and mice
41 at doses exceeding the limit dose (1000 mg/kg bw/day). There was also reduction in pituitary,
42 adrenals, thyroid and gonadal weights in a study at doses ≥500 mg/kg bw/day. However,
43 these organ changes occurred without any associated histopathological correlations.

44 Sub-chronic dermal studies in rodents did not indicate any treatment related adverse effects
45 except for a decrease in epididymal sperm density in the male mice at all doses. However,
46 similar effects were not observed in a male fertility study via dermal route in mice at doses
47 up to 400 mg/kg bw/day. Therefore, the sperm-related endpoints were either cited as
48 incidental by the authors or questioned as being insufficiently representative of historical
49 sperm counts for the rodent strain in question (SCCP, 2006). Moreover, administration of
50 dose levels up to 16-fold higher, in oral repeated dose studies did not show similar adverse
51 effect in mice.

The 2-year NTP carcinogenicity study available with BP-3 in rats showed some significant and sex-specific non-dose related effects in thyroid, adrenal glands, uterus and mammary glands. Similar observations were not made in the 2-year NTP carcinogenicity study conducted in mice. The findings in the uterus as well as in the mammary glands were viewed by the study investigators as being inconsistent, suggesting absence of an estrogenic or endocrine mode of action when compared with the effects observed with very low doses of positive control ethinyl estradiol. The endocrine-related effects reported in the above studies are not clearly identifiable as being "adverse" from a toxicological perspective.

Based on the results of the repeated dose toxicity and carcinogenicity studies available with the analogue BP-3, which considered a range of EATS-mediated and-sensitive parameters provide a coherent picture that, exposure to BP-1 is likely to also produce adverse effects only at very high doses, often concomitantly with systemic toxicity similar to BP-3.

Reproductive and development toxicity

In a poorly documented fertility study in female rats with BP-1, a NOAEL of 100 mg/kg bw/day was reported.

Further, in the continuous breeding dietary study with BP-3, which included a range of ED-mediated and sensitive parameters, showed reduced number of pups per litter and reduced dam weights in the presence of parental toxicity at doses ≥3950 mg/kg bw/day and without an effect on the average litters per pair. No adverse effects on any of the EATS-mediated parameters were observed in the 13-week male fertility study in mice. These findings therefore confirm that analogue BP-3 did not show any ED specific or EATS-mediated adverse effects in the reproductive toxicity studies in mice (see [Applicant's] Annex II; (SCCS, 2021a).

The available range of developmental toxicity studies with BP-3, which also included a range of ED-mediated and sensitive parameters, showed only adverse effects including delay in ossification and reduced pup body weights in the presence of maternal toxicity. These findings therefore confirm that BP-3 did not show any ED specific or EATS-mediated adverse effects in the development toxicity studies in rats (see [Applicant's] Annex II, (SCCS, 2021a).

Based on results of the read across studies with BP-3, a similar absence of ED specific or EATS-mediated adverse effects is expected for BP-1.

Overall evaluation of Level 4 studies by the Applicant

As no well conducted repeated dose or reproductive/development toxicity studies are available with BP-1, data for the analogue BP-3 is considered to support the ED assessment.

Overall, OECD Level 4 *in vivo* toxicity studies with BP-3 revealed potentially endocrine-related effects such as reduced sperm numbers and increased estrous cycle length in rodents at high doses in the presence of other systemic or parental toxicity. The effects observed in the 2-year NTP carcinogenicity study in rats did not show any dose response relationship and similar findings were not observed in the same study performed in mice. Further, the findings in the uterus as well as mammary glands in rats were inconsistent, suggesting an absence of an estrogenic or endocrine mode of action. The endocrine-related effects reported in the above studies are not clearly identifiable as being "adverse" from a toxicological perspective.

Taking into consideration of above information, SCCS in its final opinion has regarded the currently available evidence for endocrine disrupting properties of BP-3 as inconclusive, and at best equivocal.

Therefore, based on results of the read across studies, similar conclusions could be drawn for BP-1.

***In vivo* assays providing more comprehensive data on adverse effects on ED related endpoints over more extensive parts of the life cycle of the organism (OECD Conceptual Framework Level 5)**

No *in vivo* assays conducted prior to 2013 and providing more comprehensive data on adverse effects on ED related endpoints over more extensive parts of the life cycle of the organism were identified for BP-1.

According to the Applicant: Overall conclusion and weight-of-evidence for endocrine mediated adverse effects caused by BP-1:

Information assessing the potential endocrine disrupting properties of BP-1 is available from OECD Level 1-5 studies on BP-1 (Levels 1-4) and the analogue BP-3 (Levels 1-5).

The available weight of evidence, combining results of *in vitro* screens from ToxCast, *in vitro* and *in vivo* mechanistic testing together with *in vivo* repeated dose toxicity studies with BP-1, suggests that there is weak estrogenic and anti-androgenic activity and altered thyroid activity in the *in vitro* mechanistic studies. The estrogenic effects could be confirmed in the *in vivo* mechanistic studies, however these changes, could not be confirmed in the *in vivo* studies in rodents due to limited details.

The read-across substance BP-3 revealed either contradictory results in different studies and/or the effects were observed at relatively high doses which were consistently observed in the presence of general systemic toxicity and these doses were considered to be far beyond the human exposure range. In view of this, the SCCS considers that evidence is not conclusive enough at present to enable deriving a new endocrine-related toxicological point of departure for use in safety assessment.

Taken together and in the absence of the clear evidence for co-relating any of the adverse effects with the ED activity, which is a requirement as per the WHO definition, BP-1 is not considered to pose a hazard due to endocrine disrupting properties. Further, the selected POD (Applicant's Section **3.5**) for risk assessment is considered to be protective of the observed adverse effects on gonads and/or the reproductive parameters.

SCCS overall comment on the Potential Endocrine Activity of BP-1

According to the collective information from the published studies reported above, SCCS concludes that BP1 has estrogenic activity both *in vitro* and *in vivo*. Indeed, the available data suggest that BP1 is able to bind ER and has specific activity on both ERα et ERβ.
No anti-estrogenic, androgenic or steroidogenic activity is clearly evident, and a weak anti-androgenic activity has also been mentioned. An altered thyroid activity (TPO activity) in the *in vitro* mechanistic studies, as well as a weak binding of BP1 on ERRg, have also been reported (*in vitro* only).

From the collective evidence relating to potential endocrine effects of BP-1, the SCCS has concluded that BP-1 is an endocrine active substance. Of main concern in this regard is estrogenic activity, as it has been clearly demonstrated both *in vitro* and *in vivo.* Evidence for other endocrine-related modalities is limited to *in vitro* assays.

3.5.1. Selection of the Point of Departure (POD)

According to the Applicant, in the absence of well conducted repeated dose toxicity studies for BP-1, its systemic toxicity can be assessed on the basis of data available for the read across substance BP-3.

The submitters consider the NOAEL of 393 mg/kg bw/day from a 90-day repeated dose toxicity study in rats with BP-3 (see Section 3.4.4, Table 1) as an appropriate study to derive the critical NOAEL for risk assessment purposes. However, taking into account the recent SCCS opinion on BP-3 (SCCS, 2021a), a NOAEL of 67.9 mg/kg bw/day, derived from a pre and post-natal exposure study with BP-3 in rats, has been used as the critical NOAEL for the safety assessment of cosmetic uses of BP-1.

As discussed in [Applicant's] Section 3.3.1, the bioavailability under oral exposure conditions is assumed to be 50%, resulting in an internal dose (**Systemic POD - PODsys**) of **33.95 (rounded up to 34) mg/kg bw/day**. This value is used as a POD for MoS derivation.

Uses/exposure assessment
BP-1 is used at concentrations up to **2% in rinse-off and leave-on cosmetic formulations** (Table 11)**.** Hence, the dermal route is the major route of exposure. Accidental unintended exposure to eyes may occur but is not expected to present a significant risk. Based on eye irritation data, undiluted BP-1 is only mildly-irritating to eyes. At 2% in the product, it is not considered to alter the overall eye irritation profile of the cosmetic formulation. Oral ingestion is an unlikely route of exposure under normal and reasonably foreseeable conditions of use.

Table 11: Product types and use levels

Product Type	Product category	Product sub-types	Intended Use level
Bubble bath	Bathing, showering	Bath foam	0.1%
Bath preparations		Shower gel	0.1%
Bath soaps and detergents		Soap liquid	0.1%
Cologne and toilet waters	Fragrances	Eau de cologne	1.0%
Other fragrance preparations		Eau de toilette	1.0%
Tonics, dressings, other hair aids	Hair care	Hair styling products	0.05%
Nail creams and lotions	Nail care	Nail polish (Nail polish, base coat, and topcoat)	2%
Nail polish and enamel		Nail polish remover use quantity	1.00%
Cleansing creams	Skin care	Cleansing lotion/ make-up remover	0.10%
Other skin care preparations		Face cream	0.30%
US drug products- acne face wash		Facial cleanser	0.25%

The dermal SED calculation is based on:
• **Eproduct** – An estimated daily amount of product applied per kg body weight according to the SCCS NoG (SCCS, 2021b), (Ficheux *et al.*, 2014) and RIVM Cosmetics fact sheet (Bremmer *et al.*, 2006), considering body weight and a retention factor (0.01 or 1). Frequency is already considered in the estimated daily amount applied calculations by SCCS.

• **C**– The concentration of the substance in the cosmetic product (C) [Applicant's **Table 10**]

• **DAp** – dermal absorption = **50%**, [Applicant's **Section 3.1.1]**

Table 11 summarises the calculated SED's for BP-1 in the different product applications.

Margin of Safety (MoS) calculation
The Applicant's calculated MoS results are presented in Table below.

In accordance with the SCCS NoG, the calculated MoS for systemic toxicity should be ≥100 in order to consider a substance as safe for use.

Table 11: Systemic Exposure Doses (SED) and Margin of Safety (MoS) calculations

Product category	Product sub-types2	Use/day (g)	Retention	Eproduct (mg/kg bw day)	Max use level in finished products (%)	SED (mg/kg bw/day)	MoS
Bathing, showering	Bath foam2	17	0.01	2.83	0.10	0.0014	24286
	Shower gel1	18.67	0.01	3.11	0.10	0.0016	21250
	Soap liquid2	26.1	0.01	4.35	0.10	0.0022	15455
Fragrances	Eau de cologne2	0.65	1	10.83	1.00	0.0542	627
	Eau de toilette2	0.75	1	12.50	1.00	0.0625	544
Hair care	Hair styling products1	4	1	66.67	0.05	0.0167	2036
Nail care	Nail polish (Nail polish, base coat and topcoat) 3	-	1	1.674	2	0.017	2000
	Nail polish and Enamel2	0.2	1	3.33	1.00	0.0167	2036
Skin care	Cleansing lotion/ make-up remover2	2.5	0.1	4.17	0.10	0.0021	16190
	Face cream1	1.54	1	25.67	0.30	0.0385	883
	Facial cleanser2	2.5	0.01	0.42	0.25	0.00052	65385

Applicant's conclusion

Based on the available data, the present safety assessment supports the safe use of the BP-1 at concentration of up to 2% in cosmetic rinse-off and leave-on formulations under the conditions presented in this evaluation.

SCCS comment
As explained in sections 3.4.4, 3.4.5.2 and 3.4.6.2, the SCCS has regarded the provided evidence as insufficient to either derive a NOAEL, or to exclude mutagenicity/genotoxicity potential of BP-1 and therefore has not calculated the margin of safety. The SCCS is of the view that calculation of the margin of safety for a cosmetic ingredient is only meaningful if its mutagenicity/genotoxicity potential has been excluded.

3.4 DISCUSSION

Physicochemical properties

Benzophenone-1 (CAS No. 131-56-6, EC No. 205-029-4) is intended for use as a UV absorber and/or light-stabilizer in cosmetic products. It is described as a Light-yellow, crystalline solid that has little or no solubility in water.

Only limited data have been provided on the substance purity and the content of heavy metals and other potential impurities. Data on the substance purity, pH, and stability under use conditions in representative batches needs to be provided, along with analytical profile of impurities from the synthesis route.

Toxicokinetics

In the absence of experimental data, much of the provided information on toxicokinetic aspects has been dawn from physicochemical parameters, modelling predictions or data from the structural analogue (BP-3). BP-1 is also a known major metabolite of BP-3.

BP-1 is predicted to undergo aromatic hydroxylation and ketone reduction, and is reported to be further metabolised to 2,3,4-trihydroxybenzophenone, and conjugated with glucuronic acid and/or sulphate.

Noting the absence of relevant data on toxicokinetics for BP-1, the SCCs has accepted the Applicant’s proposed use of 50% default values for dermal absorption and 50% for oral absorption of BP-1 for use in safety assessment.

Exposure

BP-1 is intended for use in products for Bathing and showering (bubble bath, bath preparations, bath soaps and detergents); Fragrances (cologne and toilet waters, other fragrance preparations); Hair care (tonics, dressings, other hair aids); Nail care (nail creams and lotions, nail polish and enamel); Skin care (cleansing creams, other skin care preparations, US drug products-acne face wash). The SCCS has noted that the Applicant’s has used default 50% values for the calculation of both SED, and oral bioavailability.

Toxicological Evaluation

Due to gaps in the available data for BP-1, the Applicant has proposed a case for data read-across from a close analogue BP-3. This approach was used by the Applicant for data gap filling for almost all toxicological endpoints.

The justification provided for the read-across state that it has been carried out in accordance with the EChA analogue justification guidance (2017) that requires commonality between the target and the source substances in terms of functional groups and structures; structural alerts or reactivity; physicochemical properties; and the breakdown products resulting from biological/degradation processes. It has been argued that BP-1 and BP-3 share a high structural similarity (Dice index = 0.85), similarities in the key functional groups, (ketone and phenol); as well as similarities in the structural alerts identified by the profilers contained within the OECD (Q)SAR Toolbox. Also, that both substances belong to Cramer Class III, and that BP-1 is the main metabolite of BP-3.

As indicated in Preamble, the SCCS is of the view that the proposed read-across between BP-3 and BP-1 does not fulfil the critical requirements for an unbiased and transparent read-across - i.e. no details have been provided on how analogues were searched, how many were identified, and on what basis they were deselected or selected for use in the read-across. The SCCS has considered that the proposed read-across from a single analogue (BP-3) can only be considered acceptable if it is not the only evidence for a given endpoint – i.e. there is further information from other line(s) of evidence to support the read-across outcome. This means that the SCCS has only considered read-across data from BP-3 to BP-1 for an endpoint where it has formed part of a collective weight of evidence.

Irritation and corrosivity

The provided evidence includes a historic study in rabbits, and two studies in human volunteers. It is notable that the levels of BP-1 tested (1% and 0.5%) tested in the human studies is lower than the intended use level in cosmetic products (2%). However, the SCCS agrees that the available evidence suggests that BP-1 is unlikely to be a skin irritant at the proposed use levels in cosmetic products.

Also, from the two historic studies in rabbits, the SCCS has noted that BP-1 is unlikely to be an eye irritant at the proposed level of use in cosmetic products.

Skin sensitisation

The SCCS agrees that the available evidence suggests that BP-1 is not a skin sensitiser.

Acute toxicity

The limited available information from historic studies on acute toxicity in rats (oral) and rabbits (dermal) suggests that BP-1 is not likely to be acutely toxic.

Repeated dose toxicity

The data provided on repeated-dose toxicity on BP-1 is limited to an old (1968) subchronic 90-day study in rat, for which details are not available to allow assessment of reliability of the reported results. However, this study indicated depressed growth rate, quantitative changes in erythrocyte and leucocyte numbers and lesions in liver and kidney in the high dose (600 and 1900 mg/kg bw/day) group. Other studies quoted by the Applicant relate to the analogue (BP-3) and not BP-1.

In view of the questionable quality and relevance of the provided data, the SCCS considers that the available evidence does not allow derivation of NOAEL, and therefore does not agree with the proposed NOAEL of 190 mg/kg bw/day in rats from the study on BP-1.

Reproductive toxicity

The data provided on reproductive and developmental toxicity on BP-1 is limited to a fertility study in which BP-1 was administered via oral, subcutaneous and intra-peritoneal administrations to female rats for 3 days. Based on this study, a NOAEL of 100 mg/kg bw/day has been reported for reproductive effects for oral and intra-peritoneal routes, and 250 mg/kg bw/day via the subcutaneous administration (no further details). The provided information has been abstracted from CIR and EChA references, and a study report is not available to allow assessment of reliability of the study. Other studies quoted by the Applicant relate to the structural analogue (BP-3) and not BP-1. In view of the unknown quality of the study on BP-1, and questionable relevance of the information from BP-3, the SCCS considers that the proposed NOAELs for reproductive and developmental toxicity of BP-1 are not supported by the available evidence.

Mutagenicity / genotoxicity

Out of the two *in vitro* mutagenicity studies provided by the Applicant, the study on bacterial reverse mutation (Ames) test is considered by the SCCS as not valid, whereas the study on micronucleus induction in human lymphocytes as equivocal. Taken together, the provided evidence does not allow the SCCS to conclude on the safety of BP-1 in relation to genotoxicity potential.

Carcinogenicity

The SCCS has noted that the Applicant has not been able to identify any carcinogenicity studies with BP-1. Other studies quoted by the Applicant relate to the analogue (BP-3) and not BP-1. As the genotoxicity of BP-1 The SCCS is of the view that adequate evidence is not available to exclude carcinogenicity potential of BP-1.

Photo-induced toxicity

The test results from the two studies in humans have limited value for use in safety assessment, whereas the in-vitro T3T NRU study indicates absence of phototoxic potential. From the limited available information, the SCCS agrees that BP-1 is not likely to be phototoxic.

Human data

The SCCS considers Studies based on HRIPT as unethical. However, the SCCS has noted that the available information from human volunteer tests shows that BP-1 is not a skin sensitiser.

Special investigations

According to all the published studies reported above, SCCS concludes that BP1 has estrogenic activity both in vitro and *in vivo*. Indeed, the available data suggest that BP1 is able to bind ER and has specific activity on both ERα et ERβ. Neither anti-estrogenic activity, nor androgenic or steroidogenic activity is clearly evident, and a weak anti-androgenic activity has also been mentioned. An altered thyroid activity (TPO activity) in the in vitro mechanistic studies, as well as a weak binding of BP1 on ERRg have also been reported (in vitro only).

From the collective evidence relating to potential endocrine effects of BP-1, the SCCS has concluded that BP-1 is an endocrine active substance. Of main concern in this regard is estrogenic activity, and weak anti-androgenic activity, as clearly demonstrated both *in vitro* and *in vivo*. Evidence for other endocrine-related modalities is limited to *in vitro* assays.

Calculation of Margin of safety (MoS)

The SCCS is of the view that the provided evidence is not adequate to allow either derivation of a NOAEL, or exclusion of mutagenicity/genotoxicity potential of BP-1, and therefore has not calculated the margin of safety. The SCCS considers that calculation of margin of safety for a cosmetic ingredient is only meaningful if its mutagenicity/genotoxicity potential has been excluded.

4. CONCLUSION

1. *In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of Benzophenone-1, does the SCCS consider Benzophenone-1 safe when used as a light stabilizer in cosmetic products up to a maximum concentration of 2%?*

   Having considered the data provided, and the concerns relating to potential endocrine disrupting properties of Benzophenone-1, the SCCS cannot conclude on the safety of BP-1, because the information provided is insufficient to exclude genotoxicity.

   The available evidence also shows that BP-1 is an endocrine-active substance due to clear demonstration of estrogenic activity and weak anti-androgenic activity both *in vitro* and *in vivo*, and potential activity against thyroid modality *in vitro*.

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of Benzophenone-1 in cosmetic products?*

   /

3. *Does the SCCS have any further scientific concerns with regard to the use of Benzophenone-1 in cosmetic products?*

   The SCCS mandate does not address environmental aspects. Therefore, this assessment did not cover the safety of BP-1 for the environment.

5. MINORITY OPINION

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7. GLOSSARY OF TERMS

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8. LIST OF ABBREVIATIONS

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