Scientific Committee on Consumer Safety

SCCS

OPINION

on Butylated Hydroxytoluene

(BHT)

The SCCS adopted this document
by written procedure on 2 December 2021

ACKNOWLEDGMENTS

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 27 September to 23 November 2021). Comments received during this period were considered by the SCCS. For this Opinion, no change occurred.

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 BHT (Butylated hydroxytoluene), does the SCCS consider BHT safe:*

   (a) when used in mouthwash up to the maximum concentration of 0.001% and in toothpaste up to the maximum concentration of 0.1%?

   On the basis of a safety assessment, and considering the concerns related to potential endocrine disrupting properties of BHT, the SCCS is of the opinion that BHT is safe as an ingredient up to a maximum concentration of 0.001% in mouthwash and 0.1% in toothpaste.

   (a) when used in other leave on and rinse-off products up to a maximum concentration of 0.8 %?

   On the basis of a safety assessment, and considering the concerns related to potential endocrine disrupting properties of BHT, the SCCS is of the opinion that BHT is safe as an ingredient up to a maximum concentration of 0.8% in other leave-on and rinse-off products.

   BHT is also considered safe for a combined use of mouthwash at a concentration of 0.001%, toothpaste at a concentration of 0.1% and other leave-on and rinse-off products at the concentration of 0.8%.

2. *Alternatively, what is according to the SCCS the maximum concentration considered safe for use of BHT (Butylated hydroxytoluene) in cosmetic products?*

   /

3. *Does the SCCS have any further scientific concerns with regard to the use of BHT (Butylated hydroxytoluene) in cosmetic products?*

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

Keywords: SCCS, scientific opinion, Butylated hydroxytoluene (BHT), CAS No 128-37-0, EC No 204-881-4, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), scientific opinion on Butylated hydroxytoluene (BHT), preliminary version of 27 September 2021, final version of 2 December 2021, SCCS/1636/21

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TABLE OF CONTENTS

2. MANDATE FROM THE EUROPEAN COMMISSION

1. 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 specific provisions on EDs. However, it provides a regulatory framework with a view to ensuring a high level of protection of human health. Environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 ('REACH Regulation').
In the review, the Commission commits to establishing a priority list of potential EDs not already covered by bans or restrictions in the Cosmetics Regulation for their subsequent safety assessment. A priority list of 28 potential EDs in cosmetics was consolidated in early 2019 based on input provided through a stakeholder consultation. The Commission carried out a public call for data[2] in 2019 on 14[3] of the 28 substances (to be treated with higher priority-Group A substances) in preparation of the safety assessment of these substances. BHT (Butylated hydroxytoluene) (CAS No 128-37-0, EC No 204-881-4) is one of the above-mentioned 14 substances for which the call for data took place.

2. Background on BHT (Butylated hydroxytoluene)

BHT is a lipophilic organic compound. More specifically, it is a synthetic antioxidant widely used in multiple sectors, including food additives, cosmetics and personal care products, pharmaceuticals, plastics/rubbers and other petroleum products. Butylated hydroxytoluene is reducing the free-radical induced damage and spoilage; therefore, it helps maintain the properties and performance of products when exposed to air (i.e. preventing change in odour, colour, texture, etc.). BHT is reported to be used as an antioxidant at a range of concentrations (0.0002 - 0.8%) across a wide spectrum of cosmetic product types, dermally applied and sprayable products.
The ingredient BHT (Butylated hydroxytoluene) (CAS No 128-37-0, EC No 204-881-4) with the chemical name '2,6-Di-Tert-Butyl-4-Methylphenol' is not currently regulated under the Cosmetic Regulation (EC) No. 1223/2009, however it is included in the European database for information on cosmetic substances and ingredients (CosIng) with the reported functions of 'antioxidant' and 'fragrance'.
During the call for data, stakeholders submitted scientific evidence to demonstrate the safety of BHT (Butylated hydroxytoluene) in cosmetic products. The Commission requests the SCCS to carry out a safety assessment on BHT (Butylated hydroxytoluene) in view of the information provided.

Terms of reference

(1) In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of BHT (Butylated hydroxytoluene), does the SCCS consider BHT safe:

[1] https://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-739-F1-EN-MAIN-PART-1.PDF
[2] https://ec.europa.eu/growth/content/call-data-ingredients-potential-endocrine-disrupting-properties-used-cosmetic products_en
[3] Benzophenone-3, kojic acid, 4-methylbenzylidene camphor, propylparaben, triclosan, Homosalate, octocrylene, triclocarban, butylated hydroxytoluene (BHT), benzophenone, homosalate, benzyl salicylate, genistein and daidzein

i) when used in mouthwash up to the maximum concentration of 0.001% and in toothpaste up to the maximum concentration of 0.1%?

ii) when used in other leave on and rinse-off products up to a maximum concentration of 0.8%?

(2) Alternatively, what is according to the SCCS the maximum concentration considered safe for use of BHT (Butylated hydroxytoluene) in cosmetic products?

(3) Does the SCCS have any further scientific concerns with regard to the use of BHT (Butylated hydroxytoluene) in cosmetic products?

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

INCI name: Butylated Hydroxytoluene (BHT)
EC name: 2,6-di-tert-butyl-p-cresol

Ref.: Butylated Hydroxytoluene 2021a

3.1.1.2 Chemical names

IUPAC name: 2,6-di-*tert*-butyl-4-methylphenol

Other Chemical Names: 2,6-bis(1,1-dimethylethyl)-4-methylphenol, butylated hydroxytoluene; 2,6-di-tert-butyl-p-cresol (DBPC); 3,5-di-tert-butyl-4-hydroxytoluene; 1,3-di-tert-butyl-2-hydroxy-5-methyl benzene; E321,dibutylhydroxytoluene, 4-methyl-2,6-ditertbutylphenol, di-tert-butyl-methylphenol

Ref.: EDC dossier 2019; Butylated Hydroxytoluene 2021a

3.1.1.3 Trade names and abbreviations

2,6-di-tert-butyl-4-methylphenol; 2,6-di-tert-butyl-p-cresol; 4-hydroxy-3,5-di-tert-butyltoluene; Agidol 1; Antioxidant 4K; BHT, butylated hydroxy toluene; butylated hydroxytoluene; butylated hydroxy toluene; p-cresol, 2,6-di-tert-butyl-; DBPC; Dibunol; phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-; Ionol

Supplied synonyms can be found at the following link:
https://pubchem.ncbi.nlm.nih.gov/compound/Butylated-hydroxytoluene#section=Depositor-Supplied-Synonyms&fullscreen=true

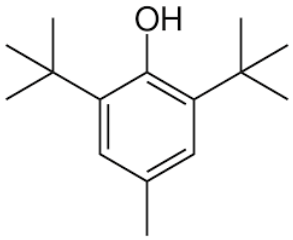
Ref.: Butylated Hydroxytoluene 2021a; ECHA 2021

3.1.1.4 CAS / EC number

CAS no.: 128-37-0
EC no.: 204-881-4

Ref.: Butylated Hydroxytoluene 2021a

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{15}H_{24}O$ or $C_6H_2(OH)(CH_3)(C(CH_3)_3)_2$

Ref.: Butylated Hydroxytoluene 2021a

3.1.2 Physical form

White to yellowish crystalline solid

Ref.: Butylated Hydroxytoluene 2021a

3.1.3 Molecular weight

220.35 g/mol

Ref.: Butylated Hydroxytoluene 2021a

3.1.4 Purity, composition and substance codes

Purity >99%

Ref.: ECHA 2021

3.1.5 Impurities / accompanying contaminants

Typical range of impurities ≤ 10 ppm heavy metals and ≤3 ppm arsenic

Ref.: Lanigan & Yamarik 2002

3.1.6 Solubility

0.4 mg/L in water at 20 °C
0.6 mg/L in water at 25 °C
1.5 mg/L at 30 ºC and 6 mg/L at 60 ºC
Freely soluble in toluene
55.9 wt% in n-heptane at 29.5 °C
34 wt% in ethanol at 28.7 °C
31.1 wt% in 1-octanol at 29.5 °C
0.5% w/w in methanol, isopropanol, methyl ethyl ketone, acetone, cellosolve, benzene, most hydrocarbon solvents, ethanol, petroleum ether, liquid petrolatum (white oil), good solubility in linseed oil.
Insoluble in propylene glycol

Ref.: Butylated Hydroxytoluene 2021a; ECHA 2021

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: 5.1

Ref.: Butylated Hydroxytoluene 2021a

3.1.8 Additional physical and chemical specifications

Where relevant:

-	organoleptic properties	faint, characteristic odour
-	melting point	70-71 °C
-	boiling point	265 °C at 760 mm Hg
-	flash point	127 °C
-	vapour pressure	0.01 mm Hg, 0.005 mm Hg at 25 °C, 0.39 Pa at 25 °C
-	density	1.05 at 20 °C
	viscosity	3.47 centistokes at 0 °C 1.54 centistokes at 120 °C
-	pKa	14, 12.2 at 20 °C
-	pH	/
-	refractive index	1.49 at 75 °C
-	topical polar surface area	20.2 $Å^2$
-	UV/visible light absorption spectrum: in dehydrated ethanol, 2 cm layer of a 1 in 100000 solution) λmax: 278 nm in isopropanol λmax: 227 nm (Log E= 3.75); 277 nm (Log E= 3.34); 283 nm (Log E= 3.34)	

Ref.: Butylated Hydroxytoluene 2021a, b; ECHA 2021

3.1.9 Homogeneity and Stability

Stable, but light-sensitive. Incompatible with acid chlorides, acid anhydrides, brass, copper, copper alloys, steel, bases, oxidizing agents. Combustible.

Ref.: Butylated Hydroxytoluene 2021b

3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS

3.2.1 Function and uses

BHT is a synthetic antioxidant used to improve the stability of cosmetic products, pharmaceuticals, fat-soluble vitamins, biomaterials, petroleum products, plastics and synthetic rubbers, and it serves as an anti-skinning agent in paints and inks. BHT is used to help preserve and stabilise the flavour, colour, freshness and nutritive value of foods and animal feed products.

BHT is used between 0.0002 and 0.8% as an antioxidant in wide spectrum of dermally applied or sprayable cosmetic product types. Low levels are used in oral care products (maximal concentration in toothpaste 0.1% and in mouthwash products 0.001%).

The presence of BHT in cosmetics may also be due to migration from packaging materials.

Ref.: EDC dossier 2019; VKM 2019

3.2.2 Dermal / percutaneous absorption

Three studies on dermal absorption cited in Lanigan & Yamarik (2002) showed absorption values ranging from 0.4% to 14.4%.

Ref.: VKM 2019

New study

Guidelines:	OECD Test Guideline 428 (2004), OECD Guidance Document No. 28, SCCS/1358/10 SCCS Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredient
Test system:	dermatomed human abdominal skin preparations (350 to 450 µm); flow-through system
Number of donors:	5 donors/timepoint; 1 cell/donor for 0.5, 1, 2, 4, 8 hours exposures (5 cells/timepoint), 1 or 2 cells/donor for 24 hours exposure (8 cells)
Membrane integrity:	TEWL between 0.7-5 g/m^2/h (closed chamber)
Test substance:	radiolabelled ([phenyl-U^{14}C]2,6-di-tert-butyl-4-methylphenol) and non-radiolabelled 2,6-di-tert-butyl-4-methylphenol 0.8% active ingredient concentration
Batch:	radiolabelled: 11311MLT001-1; non-radiolabelled: BCCC9988
Purity:	radiolabelled: 99.2% radiochemical purity, 99.10% chemical purity non-radiolabelled: 100%
Specific activity:	226.3 µCi/mg (equivalent to 0.5 µCi/cell)
Vehicle:	dicaprylyl ether, ispropyl palmitate, glyceryl stearate SE, cetaryl alcohol, dimethicone, phenoxyethanol, sodium cetearyl sulfate, carbomer, aqua
Dose applied:	5 mg/cm^2 (equivalent to 40 µg of BHT)
Exposed area:	1 cm^2
Exposure period:	0.5, 1, 2, 4, 8, 24 hours
Sampling period:	1 hour pre-dose, 0.5, 1, 2, 4 and/or 8 hours for 0.5-8 hours exposure periods; 1 hour pre-dose, 0.5 and 1 hour, then each hour for the 24 hours exposure period
Receptor fluid:	1% polyethylene glycol 20 oleyl ether in water
Solubility:	503.1 µg/mL
Recovery:	91.36±6.42%
Tape stripping:	maximum of 20 strips (pooled: 1-2, 3-8, 9-14, 15-20)
Method of analysis:	scintillation counting
GLP:	yes
Study period:	September – December 2020

Design

The preparation for the test item was applied homogeneously at 5 mg/cm² (5 mg/cell) without massage on each skin sample. The total experiment was stopped 24 hours after application. The skin was washed post-application:

- 30 minutes for 5 cells
- 1 h for 5 cells
- 2 h for 5 cells
- 4 h for 5 cells
- 8 h for 5 cells
- 24 h for 8 cells

The *stratum corneum* was taken off from the skin samples using adhesive Scotch Magic 3M® by stripping. Stripping was stopped if an epidermis/dermis separation was observed.

The two first stripped samples were analysed separately and were considered as a part of the skin which is eliminated when the user washes his hands. The strips were pooled as

follows for analysis: 1-2, 3-8, 9-14, 15-20. Using the scalpel blade, the skin corresponding to the application area was separated from the remaining (surrounding) skin. Then, the epidermis and partial dermis were separated. Samples were analysed for radiolabel content by scintillation counting. Conversion of the counts per minute (cpm) to disintegrations per minute (dpm) were performed directly by the microprocessor in the instrument using a quench curve of the appropriate scintillation cocktail stored in memory.

Results
Table 1 shows the distribution of ^{14}C-BHT.

The limit of quantitation was 100 dpm minus blank value. Results below the limit of quantitation were noted as "BLQ" in result tables and were considered as 0 for calculation.

The absorption was equal to:
- Receptor fluid + Rinsing Receptor compartment (RCR) + Epidermis + Dermis (according to the SCCS guideline)

Table 1. Distribution of ^{14}C-BHT after application to human skin (%)

6 conditions	30 min		1 h		2 h		4 h		8 h		24 h	
	n=5		n=5		n=5		n=5		n=5		n=8	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Total strips (3-20)	0.39	0.14	0.40	0.25	0.49	0.22	0.28	0.12	0.25	0.10	0.07	0.06
Skin excess including strips 1-2*	92.95	3.50	93.83	4.73	97.00	3.36	90.24	6.21	91.88	2.55	91.10	6.38
Epidermis	0.03	0.00	0.06	0.05	0.04	0.01	0.04	0.01	0.04	0.01	0.08	0.07
Dermis	BLQ	NC	BLQ	NC	BLQ	NC	BLQ	NC	0.01	0.01	0.02	0.02
Receptor fluid	0.014	0.03	0.032	0.07	BLQ	NC	0.005	0.01	0.018	0.01	0.094	0.14
Epidermis + dermis + receptor fluid**	0.04	0.03	0.09	0.07	0.04	0.01	0.04	0.02	0.07	0.020	0.20	0.20
Strips (3-20) + epidermis + dermis + receptor fluid***	0.43	0.14	0.49	0.26	0.53	0.23	0.32	0.13	0.32	0.10	0.26	0.17
TOTAL RECOVERY	93.38	3.43	94.32	4.96	97.53	3.33	90.56	6.16	92.20	2.64	91.36	6.42

*Skin excess corresponds to: Washing + Donor compartment rinsing + Remaining skin+ strips 1-2
** absorbed fraction of the applied BHT according to SCCS guideline
*** absorbed fraction of the applied BHT according to OECD guideline for the testing of chemicals: Test No.28
NC: Not Calculated
BLQ: Below the Limit of Quantification

Conclusion
The mean total recovery for each condition was within the acceptance criteria (85-115%), validating the results obtained.
The absorbed fraction of the applied BHT was low, less than 1% of the applied dose.
The absorption increased from 2 hours to 24 hours and presented the highest value for the condition at 24 hours.

Ref.: Eurofins 2020

SCCS comment
Based on Eurofins (2020) and an exposure period of 24 hours, a dermal absorption of 0.4% (mean + 1SD: 0.20±0.20%) will be used in the calculation of SED.

3.2.3 Other studies on toxicokinetics

An *in vitro* skin metabolism study was performed using excised female fuzzy rat skin and radiolabelled BHT (Bronaugh *et al*. 1989, 1990). The test compound (~5 µg BHT/cm^2 skin in 15 µl/cm^2 acetone) was applied to a 0.64 cm^2 area of dorsal skin, which had been excised, dermatomed, and placed in a flowthrough diffusion cell. The receptor fluid used was Eagle's modified minimal essential medium with 10% fetal bovine serum and the flow rate was 1.5 ml/h. The amount considered absorbed was that within the skin plus that penetrated into receptor fluid during a 24-hour period. The absorbed dose of BHT remained primarily in the skin at the end of the study. In the receptor fluid, 2.3% ± 0.1% of the applied dose that penetrated the skin was absorbed and 26.8% ± 0.2% of this was metabolised. In the skin, 11.1% ± 0.9% was absorbed and 2.4 ± 0.2% was metabolised. Combining the skin and receptor fluid, the total absorbed in this experiment after 24 hours was 13.5% of the applied dose, of which 6.6% was metabolised. The thin-layer chromatography (TLC) had two peaks of radioactivity in addition to the BHT peak: one peak chromatographed with the hydroxy-BHT standard, and the other could not be identified.

More than 40 metabolites of BHT have been empirically observed in animals following oral exposure (Matsuo *et al*., 1984; WHO JECFA, 1996). The major metabolites, and those that have been commonly seen in rats, rabbits, and humans, are shown in Figure 1. It is reported that it is not parent BHT that is responsible for any observed general toxicity but the formation of reactive quinone methide metabolites via the action of microsomal oxidation enzymes. BHT can be oxidised by cytochrome P450 (e.g. CYP2B1) to either the BHT quinone methide or the hydroxy BHT quinone methide. Multiple oxidations can occur via hydroxylations of the methyl groups on BHT, and then through subsequent conversion to the acids. These primary oxidative metabolites are most likely formed in the liver and can then be conjugated via Phase II metabolising enzymes to produce glucuronide metabolites that are excreted.

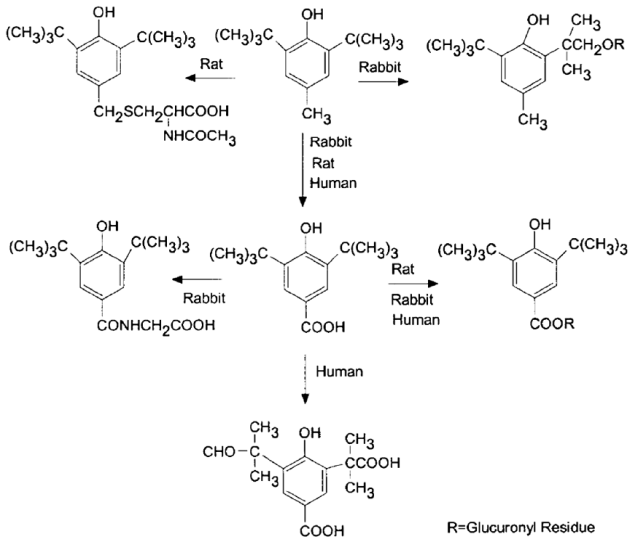


Figure 1. The major stable metabolites (from more than 40 observed) detected in urine following oral dosing of BHT in rat rabbit and human (Lanigan & Yamarik 2002).

After oral exposure there is a high bioavailability both in test animals and in humans (MAK, 2012). Upon oral absorption, BHT is generally distributed to and metabolised by the liver and is distributed to body fat. Excretion is effective, via many types of phase 2 metabolic conjugations forming tens of metabolites, and is mainly via urine and faeces in all species. There is significant enterohepatic recirculation evident in rodents that does not occur in man. The metabolism of BHT is complex and there are some relevant differences in phase 1

metabolites between species. There are also multiple detoxifying phase 2 pathways at play, all in effect leading to clearance.

The main primary metabolic pathway that is common in all species leads to the production of BHT alcohol (BHT-OH), BHT aldehyde (BHT-CHO) and BHT acid (BHT-COOH) by stepwise oxidation of the 4-methyl group. All of these are effectively conjugated and cleared. In rats and rabbits, oxidation of the p-methyl group predominates, whereas in humans the tert-butyl groups were preferentially oxidised. The key metabolite that is implicated in liver toxicity via the oral/in utero routes in rodent studies is the formation of the reactive and unstable quinone methide metabolite, which is common in all species via generic oxidation mechanisms.

In a human biomonitoring project in Germany (Murawski *et al.* 2021), quantifiable amounts of BHT acid were found in almost all samples. The geometric mean of BHT acid urinary in children and adolescents was 2.346 µg/L (1.989 µg/g_{crea}), the median (P50) was 2.18 µg/L (1.87 µg/g_{crea}), and the maximum was 248 µg/L (269 µg/g_{crea}). The median concentration was within the range of the values reported for adults and children from five different countries.
Another study in Germany among students in one community reported a median urinary BHT acid concentration of 1.06 µg/L (1.24 µg/g_{crea}) (Schmidtkunz *et al.* 2020).

Ref.: Matsuo 1984; Bronaugh 1989, 1990; WHO JECFA 1996; Lanigan & Yamarik 2002; Schmidtkunz 2020; MAK 2012; Murawski 2021

SCCS comment
As the biomonitoring data reflect aggregated exposure from all sources of BHT, without any further information about the exposure habits of the sampled people in these studies (*e.g.* diet habits, cosmetic products uses etc.), these data cannot be used directly to relate internal exposure to cosmetic uses.

3.2.4 Calculation of SED/LED

Systemic exposure dose (SED) from cosmetic use is typically based on dermal absorption data. In addition to the systemic exposure dose (SED) based on dermal absorption and on exposure through lipstick, toothpaste and mouthwash, oral exposure data also needs to be taken into account. Calculations are based on BHT concentrations as given in the mandate (0.1% in toothpaste, 0.001% in mouthwash and 0.8% in other cosmetic products).

SED calculations were based on a dermal absorption of 0.4% (mean + 1SD: 0.20±0.20%) based on Eurofins (2020) (Table 2).

Table 2. Systemic exposure doses after dermal exposure

Product category	**$E_{product}$** [1]	**Concentration of BHT**	**Dermal absorption**	**SED**
	(mg/kg bw/d)	(%)	(%)	(mg/kg bw/d)
Hydroalcoholic based fragrances	4.67	0.8	0.4	0.00015
Shower gel	2.79	0.8	0.4	0.00009
Hand wash soap	3.33	0.8	0.4	0.00011

Product category	$E_{product}$ [1]	Concentration of BHT	Dermal absorption	SED
	(mg/kg bw/d)	(%)	(%)	(mg/kg bw/d)
Shampoo	1.51	0.8	0.4	0.00005
Hair conditioner	0.67	0.8	0.4	0.00002
Body lotion	123.2	0.8	0.4	0.00394
Face cream	24.14	0.8	0.4	0.00077
Hand cream	32.7	0.8	0.4	0.00105
Deodorant non-spray	22.08	0.8	0.4	0.00071
Hair styling	5.74	0.8	0.4	0.00018
Liquid foundation	7.9	0.8	0.4	0.00025
Make-up remover	8.33	0.8	0.4	0.00027
Eye make-up	0.33	0.8	0.4	0.00001
Mascara	0.42	0.8	0.4	0.00001
Eyeliner	0.08	0.8	0.4	0.000003
	237.89			**0.00761**

[1] The specific body weight of the persons involved in the studies by Hall *et al*. (2007, 2011) is used and not the default value of 60 kg

BHT is rapidly absorbed in the gastrointestinal tract after oral exposure, and a 100% absorption of BHT was assumed (EFSA, 2012) (Table 3).

Table 3. Systemic exposure doses after oral exposure

Product category	Assumed bioavailability (%)	$E_{product}$[1] (mg/kg bw/d)	Concentration in finished product (%)	SED (mg/kg bw/day)
Toothpaste	100	2.16	0.1	0.00216
Mouthwash	100	32.54	0.001	0.00033
Lipstick	100	0.90	0.8	0.00720
Total		**35.60**		**0.00969**

[1] The specific body weight of the persons involved in the studies by Hall *et al*. (2007, 2011) is used and not the default value of 60 kg

SED for aggregated exposure is shown in Table 4.

Table 4. Systemic exposure doses for aggregated exposures

Exposure route	$E_{product}$[1]	SED
Dermal	233.22	0.00761
Oral	35.60	0.00969
Total	**268.82**	**0.0173**

[1] The specific body weight of the persons involved in the studies by Hall *et al*. (2007, 2011) is used and not the default value of 60 kg

Ref.: Hall 2007, 2011; EFSA 2012; Eurofins 2020

SCCS comment
Total SEDs after exposure to dermally applied or oral product types are 0.00746 and 0.00969 mg/kg bw/day. For aggregated exposure, SED is 0.01689 mg/kg bw/day.

3.3 TOXICOLOGICAL EVALUATION

Several evaluations and assessments of the safety of BHT have been performed. The latest are the risk assessments by the European Food Safety Authority (EFSA, 2012) and the Norwegian Scientific Committee for Food and Environment (VKM, 2019), as well as a regulatory management option analysis (RMOA) by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES 2016). EFSA (2012) includes evaluations and assessments performed by the International Agency for Research on Cancer (IARC), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Organisation for Economic Co-operation and Development (OECD) and the Scientific Committee on Food (SCF) (EFSA 2012; IARC 1987; JECFA 1996; OECD 2002; SCF 1989). VKM (2019) includes the evaluation performed by EFSA in 2012 in addition to a literature search on *in vivo* human and animal studies and *in vitro* genotoxicity studies published from 2012 to May 2018. ANSES (2016) focuses mainly on endocrine disrupting properties.

In the call for data, an additional human study (Paciencia *et al.* 2019) and four *in vitro* studies (Wada *et al.* 2004, Pop *et al.* 2016, 2018; Yang *et al.* 2018) were identified. Data from the US EPA ToxCast Endocrine Screening Program were also provided.

3.3.1. Irritation and corrosivity

SCCS comment
The SCCS concurs with the conclusions from ANSES (2016) and MAK (2012) that BHT is slightly irritating based on studies on skin and eyes of rabbits.

Ref.: MAK 2012; ANSES 2016

3.3.2 Skin sensitisation

Although the evidence on skin sensitisation in animals is limited, from a range of human experience there is no evidence to suggest that BHT is a significant human skin sensitiser or contact allergen.

A few positive patch-test reactions were considered to reflect cross-reactivity with tert-butylhydroquinone.

Ref.: le Coz 1998; Flyvholm 1990; EDC dossier 2019

SCCS comment
The SCCS agrees that the risk of skin sensitisation from current use levels is negligible.

3.3.3 Acute toxicity

3.3.3.1 Acute oral toxicity

Oral LD50 values of 1700-1970 mg BHT/kg bw in rats, 100-3200 mg BHT/kg bw in rabbits, 10 700 mg BHT/kg bw in guinea pigs, 940-2100 mg BHT/kg bw in cats, and 2000 mg BHT/kg bw in mice are indicative of low acute toxicity.

Ref.: EFSA 2012

3.3.3.2 Acute dermal toxicity

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3.3.3.3 Acute inhalation toxicity

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3.3.4 Repeated dose toxicity and reproductive toxicity

3.3.4.1 Short-term or subchronic toxicity

Short-term or subchronic exposure to BHT affects the liver of mice, rats and chicken, also showing histopathological changes in this organ. In addition, BHT has been shown to increase the relative thyroid and adrenal weight in rats. In rats, BHT given orally to male for 7 consecutive days at dose levels of 75 or 450 mg/kg bw/day induced hepatocellular proliferation, increased hepatocyte apoptosis, and elevated immunoreactivity for transforming growth factor (TGF)-β1 in the liver during the treatment, and resulted in hepatocellular inhibition of mitosis following withdrawal. EFSA concluded that none of the studies available could be used to derive a NOAEL.

Ref.: EFSA 2012; ANSES 2016

SCCS comment

In two old studies cited in ANSES (2016) (Johnson & Hewgill 1961; Gaunt *et al.* 1965), effects on adrenal weight without any histological changes were observed, whereas no effects on adrenal was observed in Price (1994). ANSES, therefore, considered that the effects of BHT on the weight of adrenals in different strains of rats are of no relevant significance.

3.3.4.3 Chronic (> 12 months) toxicity and reproductive toxicity

EFSA established an ADI for BHT of 0.25 mg/kg bw per day. The ADI was based on a NOAEL of 25 mg/kg bw per day derived from a 2-generation study in rats based on effects on litter size, sex ratio and pup body weight gain during the lactation period in the reproduction segment of the study, using an uncertainty factor of 100. This NOAEL also covers the effects on hepatic enzyme induction (and consequent thyroid hyperactivity), as well as the increase in hepatocellular adenomas and carcinomas.

In the 2-generation study by Olsen *et al.* (1986), groups of 60, 40, 40, or 60 Wistar rats of each sex (F0) were fed BHT at intake doses of 0, 25, 108, or 276 mg/kg bw/day for male rats and of 0, 26, 106 and 287 mg/kg bw/day for female rats, respectively. The F0 rats were mated after 13 weeks of treatment. The F1 groups consisted of 100, 80, 80, and 100 F1 rats, respectively, of each sex from the offspring from each group. Because of an adverse effect on the kidney in the parents, the concentration of BHT in the highest dose group was lowered to 250 mg/kg bw/day in the F1 generation. The study was terminated when rats in the F1 generation were 144 weeks of age.

The number of litters of ten or more pups at birth decreased with increasing BHT dose with the number of pups/litter amounting to 10.9, 9.6, 10.3 and 9.1* at increasing dose levels. The Armitage-Cochran test for linear trend in proportions demonstrated that the fraction of litters with ten or more pups decreased significantly with BHT dose ($p<0.001$). At weaning, treated F1 rats showed a dose dependent reduction in body weight compared to the controls. In the low, mid, and high-dose groups, the reductions in body weight were for males/females 7%/5%, 11%/10%, and 21%/16%. Food intake was comparable for all groups. (*Significant for linear trend in proportions of litters with 10 or more pups.)

Histopathological examinations indicated dose-related increases in the numbers of hepatocellular carcinomas in male rats and an increase in hepatocellular adenomas in both male and female rats. Tumours were also found in other organs of some of the treated rats, including thyroid, pancreas, ovary, uterus, thymus, reticulo-endothelial system, and

mammary gland, but their incidence was not statistically significantly different from that in controls.

In the 2-generation study by Price (1994), groups of 6 male and 48 female Wistar rats, aged 13 weeks and 9 weeks, respectively, were fed BHT in the diet at doses of 0, 25, 100 or 500 mg/kg bw/day for 3 weeks prior to mating. The litters were either culled or augmented to comprise 8 pups and were fed BHT concentrations corresponding to the diets fed to their parents, with the exception that the highest dose was reduced to 250 mg/kg bw per day. The study was terminated 22 months after the F1 male rats were placed on test diets.

In the first 5 weeks of BHT administration, a reduction in body weight gain was noted in the high-dose males. Body weight gain in all other treatment groups was similar to that in controls. At the sacrifice on day 20 of gestation, both absolute and relative liver weights of the dams were increased in a dose-related manner, statistically significant at the high dose. The body weights of the females, both including and excluding their litters, were similar in all groups.

There was a slight decrease in the numbers of pups/litter in the low and high-dose groups, but a dose-related trend was not observed. Body weights of the pups from the high-dose group were significantly lower than controls at birth (10%), and at days 6 (12%) and 21 (21%) of lactation. Mortality of the pups between culling and day 21 of lactation was 2%, 8%, 12% and 15%, in order of increasing dose. Body weights of the F1 males were lower in the high-dose group, compared with controls, throughout the 22-month treatment period. At the scheduled sacrifices, dose-related increases were observed in relative, but not absolute liver weights; the differences were statistically significant at the high dose.

A dose-related incidence of enlargement and eosinophilia of the centrilobular hepatocytes was observed at the scheduled sacrifices, starting at 6 months. This was indicative of proliferation of the smooth endoplasmic reticulum, consistent with an induction of mixed-function oxidases. Immunohistochemical staining of liver sections from control and high-dose rats revealed a marked increase in hepatocellular content and distribution of cytochrome P450 2B with BHT treatment which persisted throughout the study. Histochemical staining revealed a marked induction of gamma-glutamyl transpeptidase (GGT) activity in the periportal hepatocytes of nearly all of the high-dose rats, starting at 11 months of treatment. At 22 months, there was a higher incidence of eosinophilic and basophilic foci in the high-dose group. Histochemical staining of liver sections revealed a small number of high-dose animals with glucose-6-phosphatase-deficient AHF (altered hepatocellular foci) which was statistically significant. At 22 months, there was also a significant increase in the number of rats with hepatic nodules in the high-dose group (6/19 animals compared with none in the other groups).

Total cytochrome P450 content was increased by 30-60% in the high-dose animals starting at 21 days of age. Dose-related increases were noted in epoxide hydrolase, glutathione-S-transferase and pentoxyresorufin-O-depentylase (PROD) activities, starting at 21 days of age, which were statistically significant in the mid- and high-dose groups. The increases in PROD activity were large, 10-25 fold in the mid-dose, and 20-80 fold in the high-dose groups.

No effects on the adrenal were noted. Histopathology of the adrenal was conducted starting at 11 months post-weaning. Evidence of thyroid hyper-activity, characterised by reduction of follicular size, absence or reduction of colloid, irregularities in the follicular outline, hyperaemia and an increase in the number of follicular cells was noted starting at 11 months in both the mid-dose group (mild changes affecting 75-82% of the rats) and the high-dose group (marked changes affecting 100% of the rats). Serum thyroxin levels in treated rats did not differ from controls.

Ref.: Olsen 1986; Price 1994; EFSA 2012; ANSES 2016

The Norwegian Scientific Committee for Food and Environment performed systematic literature searches (from 2012 to 2018) to identify publications potentially indicating that

the ADI established by EFSA needed to be revised. One original study (Pop *et al*., 2013) was qualified for a risk of bias, relevance of endpoint and weight of evidence evaluation. The results reported by Pop *et al*. (2013) did not indicate that the ADI established by EFSA needed to be revised.

Ref.: Pop 2013; VKM 2019

SCCS comment

The SCCS concurs with the conclusion of EFSA (2012) and a NOAEL of 25 mg/kg bw/day, derived from two 2-generation study in rats by Olsen *et al.* (1986) based on effects on litter size, sex ratio and pup body weight gain during the lactation period in the reproduction segment of the study, will be used for MoS calculations.

3.3.5 Reproductive toxicity

See section 3.3.4.

3.3.6 Mutagenicity / genotoxicity

According to EFSA, the majority of evidence indicates a lack of potential for BHT to induce point mutations or chromosomal aberrations, or to interact with or damage DNA. Positive genotoxicity results obtained *in vitro* with BHT and BHT metabolites may be due to pro-oxidative chemistry, giving rise to formation of quinones and reactive oxygen species. Such a mechanism of genotoxicity is generally considered to have a threshold.

Two original studies on genotoxicity (Ma *et al.* 2017, Negritto *et al.* 2017), retrieved in the systematic literature searches performed by VKM, did not provide evidence of genotoxicity.

Ref.: EFSA 2012; Ma 2017; Negritto 2017; VKM 2019

SCCS comment

The SCCS concurs with the conclusions of EFSA (2012) and VKM (2019) that BHT is not of concern with regard to genotoxicity.

3.3.7 Carcinogenicity

According to EFSA, BHT in high doses can exert tumour-promoting effects in some animal models. The data do not allow the establishment of a NOAEL for a carcinogen-promotional effect. BMD analyses of the data reported by Brooks *et al.* (1976) on the incidence of lung neoplasia in mice induced by BHT revealed a BMDL10 of 38 mg/kg bw/day, and of the data reported by Olsen *et al*. (1986) on the incidence of hepatocellular carcinomas in male rats induced by BHT a BMDL10 of 247 mg/kg bw/day. The NOAEL for non-neoplastic effects in the study of Olsen *et al.* (1986) was 25 mg/kg bw/day, based on the effects on litter size, sex ratio and pup body weight gain during the lactation period in the reproduction segment of the study.

Ref.: Brooks 1976; Olsen 1986; EFSA 2012

3.3.8 Photo-induced toxicity

3.3.8.1 Phototoxicity / photo-irritation and photosensitisation

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3.3.8.2 Photomutagenicity / photoclastogenicity

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3.3.9 Human data

In Paciência *et al*. 2019, a cross-sectional analysis of 815 participants from 20 schools in Porto, Portugal, was used to assess the association between EDCs exposure and asthma, respiratory symptoms and obesity in school children. The concentrations of 13 volatile organic compounds and 2 aldehydes identified as EDCs were measured in 71 classrooms throughout 1 week. Principal component analysis (PCA) was used to assess the effect of co-exposure. Higher levels of BHT were associated with obesity.

Ref.: Paciência 2019

SCCS comment
The cross-sectional design of this study does not permit the derivation of exposure levels that could be used for risk-assessment.

3.3.10 Special investigations

3.3.10.1 Assessment of endocrine disrupting potential

Level 1: Non-test information, *in silico*, read across, *in chemico*

No data were submitted but an *in silico* assessment was performed by the SCCS through the programme VEGA QSAR. The results came out as inactive for various endocrine activity related endpoints (Table 5).

Table 5.

Estrogen Receptor binding	Relative Binding Affinity	Inactive (P)	Estrogen Receptor Relative Binding Affinity model (IRFMN) 1.0.1
Estrogenic effects	Estrogenic Receptor-mediated effect	Inactive (E)	Estrogen Receptor-mediated effect (IRFMN/CERAPP) 1.0.0
Androgenic effects	Androgenic Receptor-mediated effect	Inactive (E)	Androgen Receptor-mediated effect (IRFMN/COMPARA) 1.0.0
Thyroid alpha effects	Thyroid Receptor Alpha effect	Inactive (P)	Thyroid Receptor Alpha effect (NRMEA) 1.0.0
Thyroid beta effects	Thyroid Receptor Beta effect	Inactive (P)	Thyroid Receptor Beta effect (NRMEA) 1.0.0

P: Predicted value by the model
E: Experimental value found in the model database (hence the activity is not predicted)

Level 2: *in vitro* studies

US EPA ToxCast Endocrine Screening Program (new data released August 2019)

BHT was investigated in 26 ER-related *in vitro* tests. BHT was considered active in only 4 of these tests and in these circumstances this was only at the top dose and at a higher dose than is cytotoxic. Overall, the ToxCast Model Predictions indicate "0" (inactive) for "ER-antagonist" and "ER-agonist".

BHT was investigated in 16 test systems for androgen-related endpoints in ToxCast. BHT was considered active in only 2 of these tests and in these circumstances this was only at the top dose and at a higher dose than is cytotoxic. The ToxCast Model Predictions indicate "0" (inactive) for "AR-antagonist" and "AR-agonist".

Given the 3D structure of BHT, it is also not expected to be a substrate of the ER or AR. These results are strongly consistent with structure-activity expectations.

BHT was investigated in 11 test systems for thyroid-related endpoints in ToxCast. BHT was considered active in 5 of these tests and in 4 of these circumstances this was only at the top dose and at a higher dose than is cytotoxic. In one assay the AC_{50} is 2.38 µM. BHT was investigated in 2 test systems for steroidogenesis (aromatase-related) endpoints. BHT was considered marginally active in both assays, but only at a higher cytotoxic dose.

A borderline response was observed only on a few occasions and only at the very top dose tested above the level when cytotoxicity was apparent.

Ref.: US EPA ToxCast 2019

***In vitro* studies not included in previous risk assessments**

Four *in vitro* studies on endocrine activity, not assessed in previous risk assessments, were identified (Table 6). These studies provide only weak or no evidence for endocrine activity of BHT.

Table 6. Summary of *in vitro* studies not included in previous risk assessments

Study	Comment	Parameter
Wada *et al.* 2004	Human 293T cells	Weak estrogen-like activity in a luciferase reporter-gene assay (ca. 2 fold increase in luciferase activity reported for BHT and >10-fold increase for the positive control 17β-estradiol) No dose response
Pop *et al.* 2016	MDA-kb2 cell line	Not androgenic but weak anti-androgenicity in the presence of 5-alpha-dihydrotestosterone; DHT
Pop *et al.* 2018	Breast cancer cell line	T47D-Kbluc reporter gene assay: negative MCF-7 proliferation assay: weak anti-estrogenic activity
Yang *et al.* 2018	MVNL cell line	MVNL luciferase assay: negative
	H295R cell line	Level of estrogen in culture medium elevated

Ref.: Wada 2014; Pop 2016, 2018; Yang 2018

Level 3-5: *in vivo* assays

Several repeated dose toxicity and generation reproductive toxicity studies have been performed. These studies are summarised in Annex-1.

In the generational study by Olsen *et al*. (1986), one of the key studies used by EFSA (2012), histopathologic examinations indicated dose-related increases in the numbers of hepatocellular carcinomas in male rats and an increase in hepatocellular adenomas in both male and female rats, whereas no effect on thyroid was reported in this study. (See Section 3.3.4 for more information).

In a follow-up study by Price (1994), one of the key studies used by EFSA (2012), evidence of thyroid hyper-activity, characterised by reduction of follicular size, absence or reduction of colloid, irregularities in the follicular outline, hyperaemia and an increase in the number of follicular cells, was noted starting at 11 months at 100 mg/kg/day (mild changes affecting 75-82% of the rats) and at 250 mg/kg/day (marked changes affecting 100% of the rats). Serum thyroxin levels in treated rats did not differ from controls, and there were no thyroid-related observations in the lowest-dose group (25 mg/kg/day). Liver effects were observed at all dose levels in this study, starting at the lowest dose of 25 mg/kg/day. At 22 months, there was a higher incidence of eosinophilic and basophilic foci in the high-dose group and there was also a significant increase in the number of rats with hepatic nodules in (6/19 animals compared with none in the other groups). Dose-dependent increase in liver weight was observed at all time points with statistical significance mainly at 250 mg/kg/day. Starting at the lowest dose, a dose-related increased incidence of enlargement and eosinophilia of the centrilobular hepatocytes was observed at the scheduled sacrifices, starting at 6 months. Liver enzyme induction was investigated in detail with the following results:

- Ethoxyresorufin-O-deethylase activity (CYP1A) was statistically significantly increased on PND 20 in all dose groups (1.5, 1.5 and 1.8 fold increase for 25, 100 and 500 mg/kg/day, respectively compared to control) and on PNW 4 in the 100 and 250 mg/kg/day dose groups (1.6 and 1.7 fold increase, respectively, compared to control). The increase at termination was not statistically significant.
- Pentoxyresorufin-O-depentylase activity (PROD CYP2B) was dose-dependently increased in all dose-groups with statistical significance at 100 and 250 mg/kg/day at

all time points. The increases in PROD activity were large, 10-25 fold in the mid-dose, and 20-80 fold in the high-dose groups compared to the control.
- Epoxide hydrolase activity was increased at all dose groups at all time points with statistical significance starting for some time points in the lowest dose tested.
- Glutathione-S-transferase activity was increased at PND20 at 250 mg/kg/day and later time points at 100 and 250 mg/kg/day.

These findings indicate that liver weight increase and liver enzyme induction are the primary effects of BHT, whereas the thyroid hyper-activity was observed only at doses where liver weight and multiple liver enzymes functions were substantially increased. (See Section 3.3.4.3 for more information).

BHT has some effects on the adrenals, but they are not of relevant significance (ANSES, 2016) (see section 3.3.4.1 for more information). The effects on thyroid showed that BHT could disrupt the hormonal pathway but data are still missing to validate a mode of action and decide on the relevance of this effect for humans.

Although there are converging pieces of evidence suggesting that BHT might act on thyroid homeostasis through increased thyroid hormone hepatic catabolism, in the current knowledge, there is no direct proof that this mechanism holds true. For example, ANSES (2016) concluded that even if the studies are warnings about the potential effect of BHT to disrupt the hormonal system, the amount of information available is limited. Evaluations are based on old studies, not always available, of poor reliability, with limited reports and not statistically powerful.

Ref.: Olsen 1986; Price 1994; EFSA 2012; ANSES 2016

SCCS Overall comment on the ED Properties

Neither the *in silico* nor *in vitro* data give indication of endocrine disrupting properties of BHT. *In vivo* studies provide evidence for the liver being the primary target for BHT via the oral route, in terms of increased liver weight and increase in activities of some phase 1 and phase 2 liver enzymes at oral doses above 25 mg BHT/kg bw/day. The thyroid effects observed are likely a consequence of hepatic enzyme induction.

3.4 SAFETY EVALUATION (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

MoS calculations for separate product types and aggregated exposures are shown in Table 7. Based on effects on litter size and pup body weight gain during the lactation period, in the reproduction segment of the study Olsen *et al*. (1986; described in the EFSA 2012), the NOAEL for non-neoplastic effects was 25 mg/kg bw per day. This NOAEL also covers the observed increase in hepatocellular adenomas and carcinomas.

Table 7. MoS calculations for the different product types and aggregated exposures

Product category	**Conc. BHT** (%)	**$E_{product}$** [1] (mg/kg bw/d)	**SED** (mg/kg bw/d)		**NOAEL** (mg/kg bw/d)	**MoS**
			Dermal	**Oral**		
Hydroalcoholic based fragrances	0.8	4.67	0.00015		25	167 291
Shower gel	0.8	2.79	0.00009		25	280 018
Hand wash soap	0.8	3.33	0.00011		25	234 610
Shampoo	0.8	1.51	0.00005		25	517 384
Hair conditioner	0.8	0.67	0.00002		25	1 166 045
Body lotion	0.8	123.2	0.00394		25	6 341
Face cream	0.8	24.14	0.00077		25	32 363
Hand cream	0.8	32.7	0.00105		25	23 891
Deodorant non-spray	0.8	22.08	0.00071		25	35 383
Hair styling	0.8	5.74	0.00018		25	136 106
Liquid foundation	0.8	7.9	0.00025		25	98 892
Make-up remover	0.8	8.33	0.00027		25	93 788
Eye make-up	0.8	0.33	0.00001		25	2 367 424
Mascara	0.8	0.42	0.00001		25	1 860 119
Eyeliner	0.8	0.08	0.000003		25	9 765 625
Toothpaste	0.1	2.16		0.00216	25	11 574
Mouthwash	0.001	32.54		0.00033	25	76 829
Lipstick	0.8	0.90		0.00720	25	3 472
Aggregated exposure: dermal		**237.89**	**0.00761**		25	**3 285**
Aggregated exposure: oral		**35.60**		**0.00969**	25	**2 580**
Aggregated exposure: total		**273.49**	**0.00761**	**0.00969**	25	**1 445**

[1] The specific body weight of the persons involved in the study studies by Hall *et al*, (2007, 2011) is used and not the default value of 60 kg

3.5 DISCUSSION

Physicochemical properties
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Exposure assessment & Toxicokinetics

Based on Eurofins (2020) and an exposure period of 24 hours, a dermal absorption of 0.4% (mean + 1SD: 0.20±0.20%) will be used in the calculation of SED.

Total SEDs after exposures from dermally applied or oral product types are 0.00761 and 0.00969 mg/kg bw/day. For aggregated exposure, SED is 0.0173 mg/kg bw/day.

Toxicological Evaluation

Irritation and corrosivity
The SCCS concurs with the conclusions from ANSES (2016) and MAK (2012) that BHT is slightly irritating based on studies on the skin and eyes of rabbits.

Skin sensitisation
The SCCS agrees that the risk of skin sensitisation from current use levels is negligible.

Acute toxicity
The oral LD50 values in rats, rabbits, guinea pigs, cats and mice are indicative of low acute toxicity of BHT.

Repeated dose toxicity and reproductive toxicity
Short-term or subchronic exposure to BHT affects the liver of mice, rats and chickens. BHT has been shown to increase the relative thyroid and adrenal weight in rats. In two old studies cited in ANSES (2016) (Johnson & Hewgill 1961; Gaunt *et al.* 1965), effects on adrenal weight without any histological changes were observed, whereas no effects on adrenal was observed in Price (1994). ANSES, therefore, considered that the effects of BHT on the weight of adrenals in different strains of rats are of no relevant significance. None of the studies available can be used to derive a NOAEL.

The SCCS concurs with the conclusion of EFSA (2012) and a NOAEL of 25 mg/kg bw/day derived from two 2-generation study in rats by Olsen *et al.* (1986), based on effects on litter size, sex ratio and pup body weight gain during the lactation period in the reproduction segment of the study, will be used for MOS calculations.

Mutagenicity / genotoxicity
The SCCS concurs with the conclusions of EFSA (2012) and VKM (2019) that BHT is not of concern with regard to genotoxicity.

Carcinogenicity
According to EFSA, BHT in high doses can exert tumour-promoting effects in some animal models. The data do not allow the establishment of a NOAEL for a carcinogen-promotional effect. BMD analyses of the data reported by Brooks *et al.* (1976) on the incidence of lung neoplasia in mice induced by BHT revealed a BMDL10 of 38 mg/kg bw/day, and of the data reported by Olsen *et al*. (1986) on the incidence of hepatocellular carcinomas in male rats induced by BHT a BMDL10 of 247 mg/kg bw/day.

Photo-induced toxicity
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Human data

The cross-sectional design used in a study on association between EDCs exposure and asthma, respiratory symptoms and obesity in school children does not permit the derivation of exposure levels that can be used for risk-assessment.

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

Neither the *in silico* nor *in vitro* data give indication of endocrine disturbing properties of BHT. *In vivo* studies give evidence for the liver being the primary target for BHT via the oral route, with increased liver weight and increase in activities of some phase 1 and phase 2 liver enzymes at oral doses above 25 mg BHT/kg bw/day. The thyroid effects observed are likely a consequence of hepatic enzyme induction.

4. CONCLUSION

(1) In light of the data provided and taking under consideration the concerns related to potential endocrine disrupting properties of BHT (Butylated hydroxytoluene), does the SCCS consider BHT safe:

i) when used in mouthwash up to the maximum concentration of 0.001% and in toothpaste up to the maximum concentration of 0.1%?

On the basis of a safety assessment, and considering the concerns related to potential endocrine disrupting properties of BHT, the SCCS is of the opinion that BHT is safe as an ingredient up to a maximum concentration of 0.001% in mouthwash and 0.1% in toothpaste.

ii) when used in other leave on and rinse-off products up to a maximum concentration of 0.8%?

On the basis of a safety assessment, and considering the concerns related to potential endocrine disrupting properties of BHT, the SCCS is of the opinion that BHT is safe as an ingredient up to a maximum concentration of 0.8% in other leave-on and rinse-off products.

BHT is also considered safe for a combined use of mouthwash at a concentration of 0.001%, toothpaste at a concentration of 0.1% and other leave-on and rinse-off products at the concentration of 0.8%.

(2) Alternatively, what is according to the SCCS the maximum concentration considered safe for use of BHT (Butylated hydroxytoluene) in cosmetic products?

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(3) Does the SCCS have any further scientific concerns with regard to the use of BHT (Butylated hydroxytoluene) in cosmetic products?

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

5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

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

8. LIST OF ABBREVIATIONS

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

ANNEX 1:

Table A-1. Summary of level 3-5 *in vivo* animal studies with emphasis on effects on liver (LE), thyroid (TE) and adrenals (AE) and indicated NOAEL's (including studies with ≥2 doses only).

Reference	Species	Exposure	Doses (mg/kg bw)	liver	thyroid	adrenals	NOAEL (mg/kg bw/d)
Level 3							
Sønder gaard & Olsen, 1982[1]	Rat oral	8, 26 or 90 days	0, 25, 250		↑ uptake of ^{125}I by the thyroid at all time period studied No effects on T3 and T4 levels ↑ thyroxine half-life after 13 days returning to normal after 75 days		
Briggs *et al*. 1989	Rat m oral	highest tolerated doses for 30 days 0.5% dietary BHT		No detectable ↑ in ^{3}H-thymidine labelling Time-limited ↑ liver cell ^{3}H-thymidine labelling subsiding to control values within 8 days			
Level 4-5							
Generation Reproduction Studies							
Frawley *et al*., 1965	rat 16 m/f per dose	generation study; final sacrifice at week 42 for F0 and F1	20, 67, 200	weight ↑ serum cholesterol wk 28 ↑ histopath: no effects	no effects (weight and histopath)	no effects (weight and histopath)	67 (LE)
Olsen *et al*. 1986	rat 40-100 m/f per dose divided in subgroups	generation study; F1 up to 144 wk feeding	25, 100, 250 (in utero 500)	adenoma and carcinoma, late (≥ wk 115) significant ↑at 250 mg	no effects (tumor induction)	no effects (tumor induction)	25 (LE)
				blood cholesterol and phospholipids increased at 250 mg			
Price	rat	genera	25,	weight (rel↑),	histopath	no effects	25 (TE)

Reference	Species	Exposure	Doses (mg/kg bw)	liver	thyroid	adrenals	NOAEL (mg/kg bw/d)
1994	11-19 most m per dose per group	tion study, F1 up to 144 wk feeding	100, 250 (in utero 500)	centrilobular enlargement and eosinophilia noduli, foci at 250 mg	(hyperactivity)	(histopath)	
				enzyme induction in all dose groups	no effects (T4 induction)		
McFarlane *et al*. 1997	Rat 2M/16F F0 oral F1 lactation	Dose ranging study 14 wk	0, 500, 750, 1000	↑ weight F0 dams at ≥500			500 (LOAEL)
				At ≥750: abnormal hepatocytes (enlarged, vacuolized, proliferation of ER)			500 (maternal/fetal)
McFarlane *et al*. 1997	Rat 7M/50F F0 Oral F1 postnatal exposure,	Main study Necropsied at 22 weeks post weaning	F0 0, 25, 100, 500 F1 0, 25, 100, 250	at ≥500 F0 dams ↑ weight Abnormal hepatocytes (enlarged, vacuolized, proliferation of ER)			100 (LE)
				At ≥100 F1 pups ↑ weight abnormal hepatocytes			25 (LE)
Repeated Dose Toxicity Studies							
Sporn & Schöbesch 1961[2]	young rat 8/dose	8 wk?	20, 200	nitrogen content 'greatly reduced' at 20 mg/kg/day		non-significant increase in weight	//
Johnson & Hewgill 1961	rat weanling 3 m/f per dose 'genetic effect led to some variations in litters'	6 wk	100, 200, 300, 400, 500	weight (rel↑) (at ≥ 200 mg)		weight (rel↑, only males), histopath: no changes	100 (LOAEL, AR)
				serum cholesterol ↑ (at all doses)		adrenal cholesterol ↑ (at ≥ 300 mg)	100 (LOAEL, LE)
Allan & Engblom 1972	monkey, 2-3 per dose	4 wk gavage	50, 500	histopath (slight decrease in smooth ER, lipid droplets at 500 mg) no stat. sign. tumors in males/females	"no effect on other organs"	"no effect on other organs"	50 (LE)
				enzyme induction at 500 mg			

Reference	Species	Exposure	Doses (mg/kg bw)	liver	thyroid	adrenals	NOAEL (mg/kg bw/d)
Hiraga 1978[3]	rat 5-15 m/f per dose interim sacrifices	up to 104 wk feeding	2.5, 10, 160	weight ↑ liver weight increased (at 160 mg) no tumor induction	no clear tendency (weight, histopath)	no clear tendency (weight, histopath)	10 (LE)
NCI 1979a	rat 20-50 m/f per dose; Interim sacrifices	105 wk feeding	225, 450	weight: no data tumors: not sign.	c-cell hyperplasia in males at ≥ 225 mg; no tumor induction	Phaeochromo-cytoma in males at ≥ 225 mg – not significant and not significant for human health.	225 (LOAEL, TE)
NCI 1979b	mouse, 50 m/f per dose; interim sacrifices	107 wk feeding	m: 0, 515, 1029 f: 0, 518, 1037	weight: no data ↑ incidence of hepatocytomegaly and nonneoplastic lesions (peliosis, hepatocellular degeneration/necrosis, cytoplasmic vacuolation in males)	'no effects'	'no effects'	515 (LOAEL, LE)
Fulton *et al*. 1980	Rat 10 m	8 wk feeding	0, 30, 151, 755, 1132	Weight (abs↑ and rel↑)			30 (LOAEL, LE)
Hirose *et al*. 1981	rat 36-57 m/f per dose	104 wk feeding	125, 500	weight m (rel↑) no morphological changes	no effects (tumor induction)	no effects (tumor induction)	125 (LOAEL, LE)
				enzyme induction			
Sønder gaard & Olsen 1982[1]	rat 10-30 m per dose;	up to 90 days feeding	25, 250	weight (rel↑) at 250 mg	weight (rel↑)		25 (LE)
					no effect on enzyme act. T3/T4		250 (TE)
Shirai *et al*. 1982	mouse m/f	96wk + 8 wk plain diet	30, 150, 750	no TS related tumors			//
Furukawa *et al*. 1984[4]	rat 10 m/group	18 wk feeding	15, 50, 150, 300	weight ↑ histopath (enlargement of hep. at ≥ 150 mg)			15 (LE)
				enzyme induction (GGT and GST)			
Powell *et al*., 1986	rat 10 m per dose	28 days gavage	25, 150, 500	weight (rel↑), hepatomegaly, necrosis at 500 mg			25 (LE)
Inai *et al*.	mouse, 50	104 wk	m: 1640,	↑ weight m proliferation (foci),	not affected or	no effects on	1640 (LOAEL,

Reference	Species	Exposure	Doses (mg/kg bw)	liver	thyroid	adrenals	NOAEL (mg/kg bw/d)
1988	m/f per dose;	feeding + 16 wk plain diet	3480 f: 1750, 4230	adenoma - only males no effects in females	not examined	amyloidosis	LE)
Williams *et al*. 1990	rat males; interim sacrifices	76 wk 110 wk feeding	7.5, 23, 75, 225, 450 900 (only 110 wk)	weight ↑ (at ≥ 450mg) no morphological changes	no effects (tumor induction) no effects (tumor induction)	no effects (tumors induction) no effects (tumor induction)	75 (LE)
Tanaka *et al*. 1993	Mouse 10 m/f per dose	generation study	23, 68, 203, 608				//
Safer & Al-Nughamish 1999	rat, 10 m/dose (2/time point)	6-24 wk feeding	200, 400, 800	weight (abs↑) hypertrophy, degenerative figures no tumors			200 (LOAEL, LE)
Stierum *et al*. 2008	rat 6 m per dose	28 days feeding	28, 88, 167, 321, 1159	weight, histopath effects at 1159 mg			28 (LE)
				enzyme induction			

[1]According to the dossier, the study has severe technical limitations.
[2]According to the dossier, the study was poorly reported in Romanian.
[3]A NOAEL of 10 mg/kg bw/day was not applied by the SCCS due to the great difference between the two highest exposure doses.
[4]The report is in Japanese and the findings are poorly described in the EDC dossier. Since the SCCS have not been able to verify the results reported in the dossier, a NOAEL of 15 mg/kg bw/day will not be applied.

Ref.: Modified from EDC dossier 2019