Analysis of the most appropriate risk management option (RMOA)

Substance Name: 2,6-di-tert-butyl-p-cresol

EC Number: 204-881-4

CAS Number: 128-37-0

Authority: France

Date: March 2016

Cover Note

2,6-di-*tert*-butyl-*p*-cresol (butylated hydroxytoluene (BHT)) belongs to the broad group of alkyl phenols and as alkyl phenol ethoxylates BHT is on the Danish EPA "List of Undesirable Substances" latest updated in 2009. The group was recently reviewed in a report: "Survey of alkyl phenols and alkyl phenol ethoxylates (Danish EPA 2012). The group includes substances that are hazardous to the environment and substances that have or may have endocrine disrupting effects.

The substance is also listed in the Danish inventory of carcinogenic substances.

BHT is included in the framework on the French national Strategy on Endocrine Disruptors (Stratégie Nationale sur les Perturbateurs Endocriniens or SNPE) in 2015.
ANSES was commissioned by the French Competent Authority (Ministry of Ecology, Sustainable Development and Energy) and identified BHT as a good candidate for working further on its ED properties.

Consequently, France initiated a RMOA on BHT, mainly focused on the ED properties of this substance.

Indeed, alerts on BHT come from different sources.

BHT shares common uses with BHA, that was analysed within the framework of SNPE in 2014 and which has been identified numerous times as a potential endocrine disruptors:

- European Commission on Endocrine Disruption (EDC Database): Listed BHA as a Category 1 priority substance, based on evidence that it interferes with hormone function.
- Lately in the SIN list.

An OECD SIDS dossier was submitted in 2002 (OECD 2002). The recommendation given in the dossier is to conduct an environmental risk assessment on the substance. BHT is not readily biodegradable, and a moderate to high bioaccumulation potential has to be assumed. Furthermore, the substance has a high toxicity to aquatic organisms. No recommendations are given for human health because it is regarded as controlled in occupational settings.

BHT should not be considered as a PBT substance according to PBT Group (2004). Further testing are needed on degradation for soil and sediment in order to determine relevant degradation rates (P criteria).

BHT does not appear on the candidate list of Substances of Very High Concern (SVHC) or the Community Rolling Action Plan (CoRAP) for substances prioritised for evaluation. Furthermore no EU Member State has any intention registered on the Registry of Intention (ROI) on preparing an Annex VI (CLP) or XV dossier for identification of SVHC or for the preparation of a restriction proposal. BHT is included in the watch list of the Water Framework Directive (Commission implementing decision (EU) 2015/495 of 20 March 2015) containing 10 substances for which Union-wide monitoring data are to be gathered for the purpose of supporting future prioritisation exercises.

The assessment of the ED properties of BHT was discussed during ECHA ED-Expert Group taking place in Helsinki the 21-22th of October 2015. And the conclusions of the ED-EG are presented in section 5.2.

For the time being, the analysis of the available data leads us to propose the substance to be included in the CoRAP as soon as possible in order to clarify some uncertainties and/or to fill the gaps with reliable data.

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1 IDENTITY OF THE SUBSTANCE

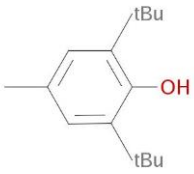
1.1 Other identifiers of the substance

Table: Substance identity BHT

EC name (public):	2,6-di-tert-butyl-p-cresol
IUPAC name (public):	2,6-di-tert-butyl-4-methylphenol
Index number in Annex VI of the CLP Regulation:	none
Molecular formula:	$C_{15}H_{24}O$
Molecular weight or molecular weight range:	220.35
Synonyms:	butylated hydroxytoluene 2,6-di-*tert*-butyl-4-methylphenol, 2,6-di-*tert*-butyl-*p*-cresol (DBPC), 3,5-di-*tert*-butyl-4-hydroxytoluene, 1,3-di-teri-butyl-2-hydroxy-5-methyl benzene E321

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



1.2 Similar substances/grouping possibilities

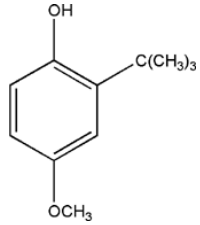
BHT and BHA (butylated hydroxyanisole) are closely related butylated compounds both used as synthetic antioxidants alone or in mixture. The uses, as well as the concerns, relating to these two substances also seem to be very similar.

EC name:	246-563-8
IUPAC name:	tert-butyl-4-methoxyphenol
Index number in Annex VI of the CLP Regulation	none
Molecular formula:	$C_{11}H_{16}O_2$
Molecular weight or molecular weight range:	180,2 g/mol
Synonyms/Trade names:	tert-butyl-hydroxyanisole; Butylated hydroxyanisole (BHA) E320

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

BHA consists of a mixture of two isomers: 3-tert-butyl-4-hydroxyanisole (3-BHA) and 2-tert-butyl-4-hydroxyanisole (2-BHA). The purity is specified to be not less than 98.5% of $C_{11}H_{16}O_2$ and not less than 85% of the 3-BHA. Therefore BHA can be considered as a monoconstituent substance.

Structural formula:



2 OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Table: Completed or ongoing processes

RMOA	☐ Risk Management Option Analysis (RMOA) other than this RMOA	
REACH Processes	Evaluation	☐ Compliance check, Final decision
		☐ Testing proposal
		☐ CoRAP and Substance Evaluation
	Authorisation	☐ Candidate List

		☐ Annex XIV
	Restri -ction	☐ Annex XVII[1]
Harmonised C&L		☐ Annex VI (CLP) (see section 3.1)
Processes under other EU legislation		☐ Plant Protection Products Regulation Regulation (EC) No 1107/2009
		☐ Biocidal Product Regulation Regulation (EU) 528/2012 and amendments
Previous legislation		☐ Dangerous substances Directive Directive 67/548/EEC (NONS)
		☐ Existing Substances Regulation Regulation 793/93/EEC (RAR/RRS)
(UNEP) Stockholm convention (POPs Protocol)		☐ Assessment
		☐ In relevant Annex
Other processes/ EU legislation		☐ Other (provide further details below)

No ongoing activity other than this RMOA.

Several public agencies such as US-EPA have identified BHA (tert-butyl-hydroxyanisole) – often claimed to be analogue of BHT - as a priority for evaluation, in particular for evaluating if it displays any ED effects. Several international and European assessments have been carried out on the BHA with regard to endocrine disruption:

[1] Please specify the relevant entry.

- The European Commission on Endocrine Disruption (EDC Database) listed BHA as a Category 1 priority substance, based on evidence that it interferes with hormone function.
- SIN List: BHA is included as endocrine disruptor with oestrogenic, thyroid and antiandrogen activity, affecting several body functions including development and reproduction.
- World Wildlife Fund 1996 lists BHA as a suspected endocrine disruptor.
- European Commission priority list 2007: BHA is in category 1 on the priority list of substances for further evaluation of their role in endocrine disruption.
- OCDE, 2010 : BHA is in the 2010 list of the high concern substances with evidence or potential evidence of ED effects, which are already regulated or being addressed under existing legislation (Dir 2002/72/EC on food Contact Materials and Dir 95/2/EC on food additives other than colours and sweeteners).
- Substance BHA evaluation has been proposed as the outcome of a French Risk Management Option Analysis Management Option Analysis after an assessment of the toxicological data in the dossier and following a discussion with experts of the ED Expert Group of ECHA after an assessment of the toxicological data in the dossier and following a discussion with experts of the ED Expert Group of ECHA in 2014.
- BHA was proposed to be put under targeted substance evaluation in particular for endocrine disruption properties both in Human Health and Environment. BHA has been included in the CoRAP and evaluated in 2015.

In order to check if a read-across is plausible between BHA and BHT, ANSES have performed a comparison of structure-activity between BHA and BHT.

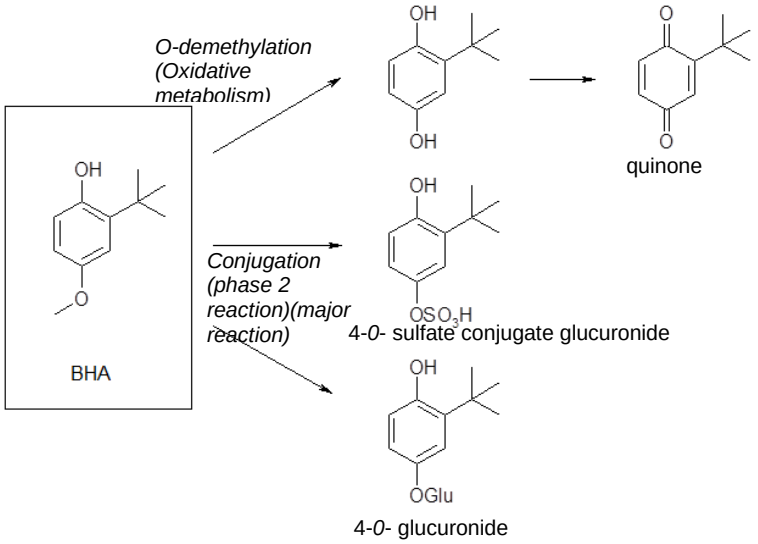
The phenol group of BHT is protected by the two butylated groups when the phenol of BHA is more accessible. The methoxy group in BHA molecule is relatively stable, the reaction of the molecule is more probable and the transformation in quinone can ocurr. Therefore, polarity and reactivity of BHA and BHT are different.

Available data on Absorption, Distribution, Metabolism, Excretion endpoints for BHA and for BHT show differences:

BHA is absorbed and rapidly excreted by the rat, rabbit and man, with little evidence of long-term tissue storage. The major metaboblic pathways are conjugation (phase 2 reactions) in all species: rat, rabbit, man, and the conjugation with glucuronic acid predominates; oxidative metabolism (O-demethylation) being relatively unimportant. In dog, absorption and urinary excretion is lower, and oxidative metabolism is more important than in other species (see the metabolic pathways scheme above).

Metabolites were identified: the principal metabolic pathway for BHA in all species studied is conjugation of the free hydroxyl group with both glucuronic acid and sulphate. In rat, rabbit and man, conjugation with glucuronic acid predominates, whereas in the dog, sulphation is the major reaction. In rat, the 3-isomer is excreted principally as the glucuronide conjugate, whereas the 2-isomer is excreted as the sulphate conjugate. *O*-Demethylation is a minor pathway in rat, rabbit and dog.
Recent studies in man have suggested the formation of a *tert*-butylhydroquinone by *O*-demethylation, followed by conjugation with glucuronic or sulphuric acid (EI-Rashidy and Niazi, 1980).
Studies with rat liver microsomes and 3-BHA demonstrated the formation of *3-tert-butyl-4,*5-dihydroxyanisole and the butylated hydroquinone and led to the

suggestion that the quinone could be formed (Armstrong and Wattenburg, 1985). This was confirmed by Cummings, Ansari, Guengerich *et al* (1985).

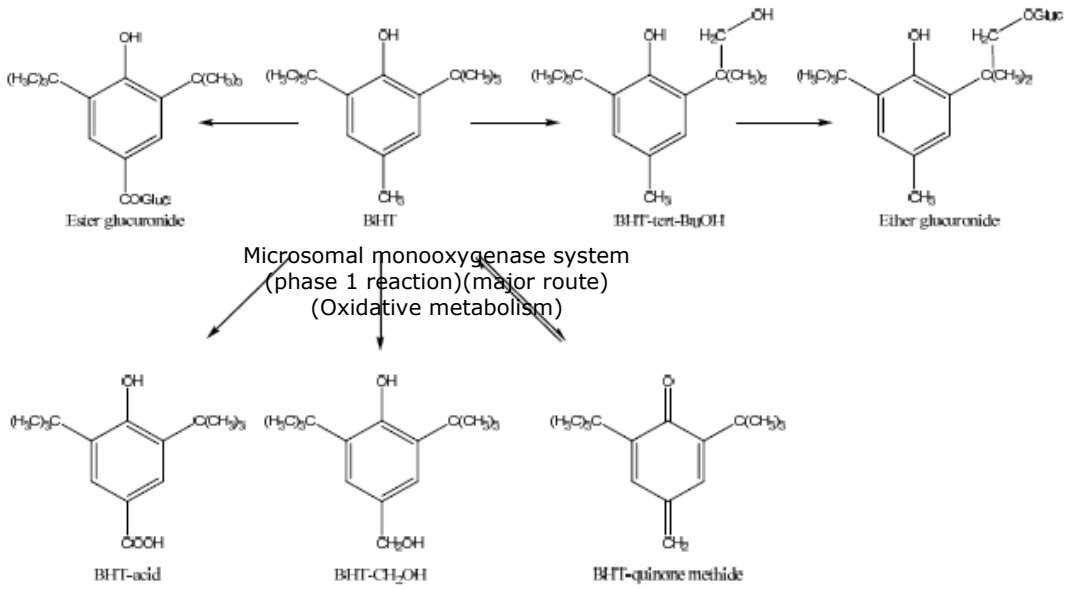


In contrast, BHT is cleared less rapidly from most species, enterohepatic circulation being partly responsible for the delay. Tissue accumulation is also greater for BHT than for BHA. It is excreted mainly via urine and feces. There is evidence of accumulation in liver and body fat. Oxidative metabolism (phase 1 reactions) mediated by the microsomal monooxygenase system is the major route for BHT degradation (Conning D.M, and Phillips J.C, 1986[2], Thompson, 1987[3]) : Oxidation of the ring methyl group in rat, rabbit, monkey and Oxidation of the *tert*-butyl groups in man.

Metabolites were identified: in the rat, the major urinary metabolites were 3,5-di-tert-butyl-4-hydroxybenzoic acid (BHT-acid), both free and as the ester glucuronide, and S-(3,5-di-tert-butyl-4-hydroxybenzyl)-N-acetyl-cysteine (BHT mercapturic acid). The major faecal metabolite was free BHT-acid. In rabbit, BHT-acid was also the major metabolite of BHT. In monkey, the major metabolite was the ester glucuronide of BHT-acid. In man, the major metabolite is on the form of an ether-insoluble glucuronide of an oxidized derivative of BHT, later identified as 5-carboxy-7-(1-carboxy-1-methylethyl)-3,3-dimethyl-2-hydroxy-2.3-dihydro-benzofuran in 1978.

[2] Conning D.M., Phillips J.C., Comparative metabolism of BHA , BHT and other phenolic antioxidants and its toxicological relevance. Fd Chem. Toxic. Vol. 24, No. 10/11, pp.1145-1148, 1986.

[3] Thompson, J. A., A. M. Malkinson, M. D. Wand *et al*. 1987. Oxidative metabolism of butylated hydroxytoluene by hepatic and pulmonary microsomes from rats and mice. *Drug Metab. Dispos.* 5:833–840.



The comparison of ADME properties revealed some differences in their absorption and metabolic disposition: BHT is metabolized by oxidation reaction and it is an inducer of the microsomal monoxygenase system, BHA is only a weak inducer (Conning D.M, and Phillips J.C, 1986). The authors Oikawa *et al*. in 1998 suggested that following metabolic conversion BHT may induce oxidative damage to DNA through two different pathways, i.e. the oxidation by BHT-OOH in presence of transition metals, and the intracellular generation of H_2O_2 by BHT-quinone.

As a consequence BHA database has not been taken into account while evaluating BHT effects.

2.1 Other Relevant EU legislation for the substance

The table below indicates for each known use of BHT which one is already regulated by specific EU legislation.

Different uses of BHT	**Non REACH regulations**	
Food products or feedingstuffs (food additive)	Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC)) No1333/2008	BHT is an authorised synthetic antioxidant preservative that was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the latest in 1996 and the EU Scientific Committee for Food (SCF) in 1987 (European Parliament and Council Directive 95/2/EC (1995) on food additives other than colours or sweetener) then reevaluated by Efsa in 2012. Maximum level of 100 mg BHT (E321)/ kg in oils and fats. BHT is a synthetic antioxidant

		authorised for use in fats and oils, only for the professional manufacture of heat-treated food, in frying oil and frying fat (excluding olive pomace oil) and in lard, fish oil, beef, poultry and sheep fat. It is permitted alone or in combination with other antioxidants such as gallates, tert-butylhydroquinone (TBHQ) and butylated hydroxyanisole (BHA) in amounts up to 100 mg/kg expressed as fat.
Food products or feedingstuffs (food additive)	Regulation (EC) No 95/2/EC	In addition, BHT is permitted in chewing gum alone or in combination with the aforementioned antioxidants at a maximum level of 400 mg/kg chewing gum (Directive No 95/2/EC).
Food products in animal nutrition	Regulation (EC) No 1831/2003	BHT is authorized in feed product for animal nutrition, with a maximal concentration set at 100 mg/kg.
Food contact material	Regulation (EC) No. 1935/2004	BHT is authorized in food contact material (packaging material for fat containing foods).
Cosmetics	EU Cosmetic Products Regulation (EC) No 1223/2009	BHT is listed in the EU database of cosmetic ingredient (CosIng) for its functions as a maskant and antioxidant.
Pharmaceuticals	Regulation (EC) No726/2004	BHT is listed in the list of excipients in medicines with notable effects.
Directive on Chemicals Agents at Work	Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work	No SCOEL recommendation regarding indicative OEL values is available. National OEL value at 10 mg/m^3 for an 8-hours work day has been adopted by Germany, Finland, France, Austria, UK and Denmark.
Waste Framework Directive	Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste	BHT in waste at a concentration that triggers classification of a mixture according to the CLP Regulation will render the waste hazardous.
Water Framework	Commission	BHT is included in the watch list of

Directive	implementing decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 200/105/EC	the Water Framework Directive containing 10 substances for which Union-wide monitoring data are to be gathered for the purpose of supporting future prioritisation exercises. In fact for these substances the information available indicated that they may pose a significant risk, at Union level, to or via the aquatic compartment, but monitoring data are insufficient to come to a conclusion on the actual risked posed. The analytical methods indicated for BHT are solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC/MS) and the maximum acceptable method detection limit of 3.16 µg/L.

3 HAZARD INFORMATION (INCLUDING CLASSIFICATION)

3.1 Classification

3.1.1 Harmonised Classification in Annex VI of the CLP

Table: Harmonised classification

Index No	International Chemical Identification	EC No	CAS No	Classification		Spec. Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)		
No current entry							

3.1.2 Self classification

- In the registration dossier:

In its registration dossier, the lead registrant classifies BHT as Aquatic Chronic of category 1 only.

- The following hazard classes are in addition notified among the aggregated self classifications in the C&L Inventory:

Hazard Class and Category Code(s)	Hazard Statement Code(s)	Number of Notifiers
Acute Tox. 4	H302	321
Acute Tox. 4	H312 (dermal)	93
Acute Tox. 4	H332 (inhalation)	35
Acute tox 3	H310	1
Skin Irrit. 2	H315	259
Eye Irrit. 2	H319	322
Skin Sens. 1	H317	35
Resp. Sens 1	H334	1
STOT SE 3	H335	125
Mut. 1B	H340	9

Mut.2	H341	3
Carc. 2	H351	10
Carc. 1B	H350	1
Repr. 2	H361	10
STOT SE 1	H370 (nervous system)	38
STOT RE2	H373 (lung, liver)	35
STOT RE2	H373 (liver)	39
Aquatic Acute 1	H400	1718
Aquatic Chronic 2	H411	6
Aquatic Chronic 1	H410	37
Aquatic Chronic 4	H413	293

3.1.3 Proposal for Harmonised Classification in Annex VI of the CLP

There is no current proposal for classification nor any intention indicated in the Registry of intentions.

The data presented in section 3.2 here below might warrant discussion at the RAC level respectively for carcinogenic and reprotoxic effects of BHT, at least for having identical classification & labelling proposed. Indeed, given the heterogenicity of the self classifications, it worth's proposing an harmonized classification as a risk management option.

3.1.4 CLP Notification Status

There are 89 aggregated notifications by the 25 August 2015 containing a total of 4188 notifications.

Table: CLP Notifications

	CLP Notifications[4]
Number of aggregated notifications	89
Total number of notifiers	4188

[4] C&L Inventory database, http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database (accessed 25 August 2015)

3.2 Additional hazard information

Hazards properties presented in this section are mainly based on previous European evaluations.

Numerous investigations on various endpoints of the toxicity of 2,6-di-*tert*-butyl-*p*-cresol (BHT) have been carried out. In a review published in 2002, many aspects of the toxicity of BHT have been described in detail (Lanigan *et al.*, 2002[5]). BHT was also assessed in detail by OECD 2002.
Further comprehensive reviews and detailed assessments can be found in BUA Report 58 (BUA, 1991) and the relevant Supplementary Report (BUA, 2000), in Williams *et al*. 1999 and in WHO, 1996 and JECFA, 1996. Only the studies relevant for the present assessment will be described in more detail below.

- Lanigan *et al.,* 2002. Final report on the safety assessment of BHT. *International Journal of Toxicology,* 21(Suppl. 2):19–94, 2002.
- OECD, *2,6-di-tert-butyl-p-cresol (BHT) (CAS No : 128-37-0).* OECD Screening Information Data Sets (SIDS). Orlando (Floride) : UNEP Publication. (2002).
- BUA (GDCh-Advisory Committee on Existing Chemicals) *(*2000*) BUA Report* 219*, Supplementary Report* VI*, S. Hirzel Verlag,* Stuttgart.
- Williams GM*,* Iatropoulos MJ*,* Whysner J *(*1999*).* Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives*. Food Chem Toxicol* **37***:* 1027–1038.
- WHO (JECFA), 1996. 833. Butylated hydroxytoluene. Toxicological evaluation of certain food additives and contaminants in food. Prepared by the forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Safety evaluation of certain food additives. WHO Food Additives Series 35*, World Health Organization,* Geneva, Switzerland. http://www.inchem.org/documents/jecfa/jecmono/v35je02.htm.

BHT has recently been evaluated by the EFSA panel in relation to the use as a food additive and nutrient sources added to food (ANS):

- EFSA Scientific Opinion on the re-evaluation of butylated hydroxytoluene BHT (E 321) as a food additive. EFSA Journal 2012;10(3):2588[43 pp.]. doi:10.2903/j.efsa.2012.2588

Several international and European assessments have been carried out on toxicological effects for carcinogenicity potential and endocrine disruptor potential of BHT in comparison to its analogue for uses BHA.

Skin Sensitization

BHT is identified as skin sensitizer in the 2 following reports:

- U.S. National Library of Medicine, in *Haz-Map: Occupational Exposure to Hazardous Agents*, 2010, http://hazmap.nlm.nih.gov. BHA and BHT can produce skin allergic reactions.

- SCCS (Scientific Committee on Consumer Safety), opinion on fragrance allergens in cosmetic products, 26-27 June 2012, http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_102.pdf.

[5] Lanigan *et al* 2002. Final report on the safety assessment of BHT. *International Journal of Toxicology*, 21(Suppl. 2):19–94, 2002.

On the basis of the animal and clinical data included in its report, the CIR Expert Panel concludes that BHT is safe as used in cosmetic formulations (CIR[6] expert panel, «Final report on the safety assessment of BHT.» *International Journal of Toxicology.* Vol. 21, no. suppl. 2, p. 19-94. (2002)).

As far as regulatory concern, the use of BHT in cosmetics is not regulated in Canada, although Health Canada has attributed to BHT "moderate health priority".

Carcinogenicity

- *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans vol. 17* (Paris : Centre international de Recherche sur le Cancer), vol. 40 (1986) classified BHA as potentially carcinogen for human.

- *WHO (JECFA) 1996*. 833. Butylated hydroxytoluene. Toxicological evaluation of certain food additives and contaminants in food. Prepared by the forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Safety evaluation of certain food additives. WHO Food Additives Series 35*, World Health Organization,* Geneva, Switzerland.

- *EFSA 2012.* Scientific Opinion on the re-evaluation of butylated hydroxytoluene BHT (E 321) as a food additive. EFSA Journal 2012;10(3):2588[43 pp.].

Endocrine disruptor and reprotoxic effects

- PNUE et OCDE, *2,6-di-tert-butyl-p-cresol (BHT) Screening Information Data Set: Initial Assessment Report* (Paris : PNUE, 2002), http://www.inchem.org/documents/sids/sids/128370.pdf. A long-term exposure to high doses of BHT is toxic to mice and rats, causing problems to thyroid and also other long terms effects on liver, and kidney. It also interfers with the functioning of the lungs and the blood clotting.

- TEDX list of potential Endocrine Disruptor : Hughes *et al.,* 2000. Estrogenic alkylphenols induce cell death by inhibiting testis endoplasmic reticulum Ca(2+) pumps, *Biochem Biophys Res Commun*. 2000 Nov 2;277(3):568-74. Industrial estrogenic alkylphenols such as BHT may disrupt testicular development by inhibiting ER Ca(2+) pumps, thus disturbing testicular Ca(2+) homeostasis and decrease male fertility using an immunohistochemical method.

- Some limited data in two *in vitro* studies suggest that high doses of BHT can simulate estrogen (Wada H. *et al*., 2004[7]), sexual primary female hormone, as well as preventing the expression of male sex hormones,

[6] CIR : Cosmetic ingredient review

[7] Wada, H. *et al*., 2004. «In vitro estrogenicity of resin composites», *Journal of Dental Research* 83, no. 3 (March 2004) : 222-6.

which would result in adverse effects on reproduction (Schrader TJ et Cooke GM, 2000[8]).

3.2.1 Health hazards related to butylated hydroxytoluene

BHT was reviewed under the OECD Cooperative Chemicals Assessment Programme in 2002, and as no additional information was provided in the CSRs compared to the OECD dossier and the EFSA panel, most of the conclusions were proposed in accordance with the SIDS (SIAM 14, 26-28 March 2002) and the EFSA panel (2012).

3.2.1.1 Toxicokinetics

Absorption, distribution, metabolism and excretion of BHT have been studied in mice, rats, rabbits, chickens, monkeys and humans.

As discussed and concluded in the SIDS Initial Assessment Report, BHT is rapidly absorbed through the gastrointestinal tract and, to a small extent, through the intact skin. BHT is cleared less rapidly than BHA from most species, enterohepatic circulation being partly responsible for the delay. In humans, in contrast to rats, no considerable enterohepatic circulation was shown (WHO, 1996).

Upon absorption, BHT is well distributed to the liver and body fat.

Excretion is mainly *via* urine and faeces. Tissue accumulation is also greater for BHT than for BHA.

The metabolism of BHT is complex. There may be important species differences. Oxidative metabolism (phase 1 reactions) mediated by the microsomal monooxygenase system is the major route for BHT degradation (Lanigan *et al.,* 2002 [3]; Thompson *et al.*, 1987[9]); oxidation of the ring methyl group predominates in the rat, rabbit and monkey, and oxidation of the tert-butyl groups in man. BHT is metabolized by oxidation reactions and is an inducer of the microsomal monooxygenase system.

BHT does penetrate the skin, but the relative low amount absorbed remains primarily in the skin (Lanigan *et al.,* 2002).

3.2.1.2 Acute toxicity

BHT has a low acute toxicity (WHO 1996). There were no specific clinical symptoms in mammalians (Madhavi *et al* 1996[10]). In rat, the oral LD50 was > 2930 mg/kg bw, the LD50 after dermal exposure was > 2000 mg/kg bw. In

[8] Schrader, TJ et GM Cooke, 2000. «Examination of selected food additives and organochlorine food contaminants for androgenic activity in vitro», *Toxicological Sciences* 53, no. 2 (February 2000) : 278-88.

[9] Thompson, J. A., A. M. Malkinson, M. D. Wand *et al*. 1987. Oxidative metabolism of butylated hydroxytoluene by hepatic and pulmonary microsomes from rats and mice. *Drug Metab. Dispos.* 5:833–840.

[10] Madhavi, D., Deshpande, S. and Salunkhe, D., 1996. Food Antioxidants: Technological, Toxicological, and Health Perspectives. Marcel Dekker, New York.159-265.

rabbit, the oral LD50 was 10700 mg BHT/kg bw. In guinea-pig, the oral LD50 was 10700 mg BHT/kg bw. In mouse, the oral LD50 was 2000 mg/kg bw.

Shilian and Goldstone (1986)[11] reported a human case of gastritis caused by ingestion of BHT in a 22-year-old woman who ingested 4 g of BHT on an empty stomach two days before the onset of the gastritis (corresponding to an acute dose of about 67 mg/kg bw assuming a body weight of 60 kg). Later that evening she experienced severe epigastric cramping, generalized weakness, nausea and vomiting, followed by dizziness, confusion and a brief loss of consciousness. A similar case study was reported by Grogan (1986)[12]. A 24-year-old woman complained of light-headedness, unsteadiness of gait and slurred speech. On examination the following findings were noted: dysarthria, wide-based gait, a positive Romberg test, slowed mentation without thought disorder and dysmetria of the left (non-dominant arm). On the evening before admission the patient ingested 80 grams of BHT suspended in safflower oil on an empty stomach (dose equivalent to about 1.3 g/kg bw, assuming a 60 kg body weight).

BHT has a low acute toxicity in animals. Some effects of BHT were reported in human cases and caused some neurological disturbances following oral intake. These effects will be further evaluated during substance evaluation.

3.2.1.3 Irritation to eye and skin

BHT was slightly irritating to the skin and eyes of rabbits (Bomhard, E., 1996b[13] , OECD 2002).

3.2.1.4 Hypersensitivity, allergy and intolerance

Studies in Humans

The Scientific Committee on Consumer Safety (SCCS) reported in a recent opinion on fragrance allergens in cosmetic products (2012) the following statement "As antioxidants are now frequently used at elevated concentrations in scented products due to a growing awareness of the problem of autoxidation, there is a risk that sensitisation caused by the antioxidants will rise. One of the most used antioxidants is butylated hydroxytoluene (BHT) which is considered a minimal risk for sensitisation in the concentrations used but nevertheless, with increased concentrations and usage, the risk of sensitisation could increase".
Despite of being in wide dispersive use for years, only a few cases of skin sensitization due to BHT as ingredient of various products are reported.

Some reports showed some evidence of skin sensitization potential of BHT following oral intake. A sometimes pronounced urticarial reaction was elicited by oral provocation with BHA and BHT among patients with chronic urticaria (Roed-

[11] Shilian DM and Goldstone J, 1986. Toxicity of butylated hydroxytoluene. *The New England Journal of Medicine* 314(10), 648-649.
[12] Grogan MW, 1986. Toxicity from BHT ingestion. *The Western Journal of Medicine* 145(2), 245–246.
[13] Bomhard E *(*1996*)* Acute toxicological evaluation of butylated hydroxytoluene*. J Am Coll Toxicol* **15** *:* S72.

Petersen and Hjorth 1976[14]). 112 patients with eczematous dermatitis were patch tested with 2% BHT or BHA in petrolatum, 2 reacted to both antioxidants, 1 reacted to BHT alone, and 1 reacted to BHA alone. The patients who reacted to both were asymptomatic when the antioxidants were added to food, and both had acute flares of vesicular eczema on the fingers after oral administration of small amounts. In another study, 83 patients did not react after treatment with 5% BHT or BHA in alcohol.

Goodman *et al*. reported in 1990[15] the case of two patients with chronic idiopathic urticaria who were subjected to double-blind, placebo-controlled, oral challenges with a series of food additives. During testing, BHT and BHA were identified as causative agents. Avoidance of foods containing BHT and BHA resulted in long-term reduction in severity and frequency of urticarial episodes. In a double-blind placebo controlled study by Hannuksela and Lahti in 1986[16] with challenge tests of 44 patients with chronic urticaria, 91 with atopic dermatitis and 123 with contact dermatitis, none reacted to BHT when it was ingested in a capsule containing 50 mg BHT.

When patch-tested on more than 15 individuals, BHT showed mild skin irritation. A positive skin reaction 14 days later was interpreted as sensitization (Mallette and von Haam, 1952[17]). However, these limited reports do not allow drawing any conclusions as to the skin irritation and sensitization of BHT in view of the widespread exposure to BHT in consumer products. More recent patch test results obtained from the medical surveillance of great numbers of workers (de Boer *et al*., 1989[18], Flyvholm and Menne, 1990[19]) or patients (Kanerva *et al*., 1997[20]; 1999[21]) were all negative. As conclusion some patch tests revealed positive results for BHT but most of them are negative.

Studies in Animals

There are no relevant experimental data available. Limited studies with guinea pigs showed no indications of a sensitizing potential. In a very limited study carried out in 5 guinea pigs, the intradermal challenge (0.04 mg BHT/animal) elicited no signs of sensitization after intradermal injection (3×/week 0.04 mg BHT/animal in 0.05 ml 10% ethanol or 0.08 mg BHT/animal in 0.1 ml 10% ethanol; total of 10 applications) (Deichmann *et al.,* 1955).

[14] Roed-Petersen, J. et Hjorth, N., «Contact dermatitis from antioxidants - Hidden sensitizers in topical medications and foods.» *British Journal of Dermatology.* Vol. 94, no. 3, p. 233-241. (1976).

[15] Goodman DL, McDonnell JT, Nelson HS, Vaughan TR and Weber RW, 1990. Chronic urticarial exacerbated by the antioxidant food preservatives, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). *Journal of Allergy and Clinical Immunology* 86, 570-575.

[16] Hannuksela M and Lahti A, 1986. Peroral challenge tests with food additives in urticaria and atopic dermatitis. International *Journal of Dermatology* 25, 178-180.

[17] Mallette FS, von Haam E, 1952. Studies on the toxicity and skin effects of compounds used in the rubber and plastics industries. I. Accelerators, activators, and antioxidants. American Medical Association Archives of Industrial Hygiene and Occupational Medicine 5, 311–317.

[18] de Boer EM, van Ketel WG, Bruynzeel DP, 1989. Dermatoses in metal workers. (II). Allergic contact dermatitis. *Contact Dermatitis* 20, 280–286.

[19] Flyvholm MA and Menne T, 1990. Sensitizing risk of butylated hydroxytoluene based on exposure and effect data. Contact Dermatitis 23, 341–345.

[20] Kanerva L, Jolanki R, Estlander T *(*1997*)* Allergic and irritant patch test reactions to plastic and glue allergens*. Contact Dermatitis* 37*:* 301–302.

[21] Kanerva L, Jolanki R, Alanko K, Estlander T *(*1999*)* Patch-test reactions to plastic and glue allergens*. Acta Derm Venereol* 79*:* 296–300.

There are no data available for the sensitizing effect of BHT dusts or vapours on the respiratory tract.

The limited reports on human do not allow any conclusions to be drawn about sensitization to BHT following oral intake and there are some doubts about skin sensitization potential to BHT. Further data might be necessary to clarify this endpoint.

3.2.1.5 REPRODUCTION/DEVELOPMENTAL TOXICITY

Fertility

No indications of an impairment of fertility even at high doses are obtained from the overview of the available studies which are numerous, most of them are older one- and multi-generation studies in mice and rats.

A number of studies showed no consistent dose-related effects on reproductive parameters in rodent : mice (Clegg 1965[22], Hiraga, 1978[23]) at doses up to 800 mg/kg bw/d, in hamster at doses up to 280 mg/kg bw/d and in rat at doses up to 750 mg/kg bw/d (Clegg 1965, Han *et al*.,1993[24]).

MICE study Reproduction	**Observed effects**
Johnson, 1965[25] outbred albino mice	At 0.5% level of BHT(750 mg/kg bw/d): increase in the length of time to birth and at 12 days after birth. Mean number of pups alive, mean pup weight and mean total litter eight lower than the overall average.
Stokes and Scudder, 1972, 1974 [26] Swiss-Webster	At 714 mg/kg bw/d : F1 : Behavioural changes of offsprings (decreased sleeping, increased social and isolation-induced aggression and a severe deficit in learning). No data on prenatal toxicity parameters or maternal toxicity.
Tanaka *et al*. 1993[27]	At 610 mg/kg bw/d :

[22] Clegg, DJ, 1965. An Absence of Teratogenic Effect of Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) in Rats and Mice. *Fd Cosmet. ToxicoL* Vol. 3, pp. 387-403.

[23] Hiraga, K. (1978). Life-span oral toxicity study of 3,5-di- *tert*-hydroxytoluene (BHT) in rats. *Ann. Rep. Tokyo Metropolitan Research Lab. Public Health,* 32: 83.

[24] Han SY, Kim PG, Park KL, Shin JH, Lee YM, Kwon SC, Kim JG, Ryu HY, Lee JJ, Kang MO, Jang SJ, Hong YT *(*1993*)* A teratogenicity study on phenolic antioxidants in rats*. Teratology* 48: 507.

[25] Johnson AR, 1965. A re-examination of the possible teratogenic effects of butylated hydroxytoluene(BHT) and its effect on the reproductive capacity of the mouse. *Food and Cosmetics Toxicology* 3,371-375.

[26] Stokes JD and Scudder CL, 1974. The effect of butylated hydroxyanisole and butylated hydroxytoluene on behavioral development of mice. *Developmental Psychobiology 7*, 343-350.

CD-1	F1: decrease in body weight gain from day 7 to 21 of lactation, but no body weight differences in the F2 pups compared with controls in F1. male F2 : 180°rotation decreased (PND21, at all doses but no dose relation). No overt effects on reproduction reported.

Two 2-generation rat studies were reported: One study published by Olsen *et al.* (1986) and the other study is an unpublished report from The Robens Institute (Price, 1994) included in the JECFA evaluation published in 1996 and also submitted to EFSA after a public call for data in 2012.

2-generation reproductive toxicity study	**Observed effects**
Olsen *et al.* (1986)[28] (published) *in utero* and 144 weeks exposure Wistar rats ♂/♀ (F0) 0; 25; 100; 500 mg/kg bw/day (F1) 0; 25; 100; 250 mg/kg bw/day	<u>Parental toxicity</u> At Birth decrease in mean number of pups per litter with increasing BHT dose (10.9 ; 9,6; 10,3; **9,1*** (*significant for linear trend in proportions of litters with 10 or more pups) <u>Reproduction:</u> F1 : at weaning dose-dependent reduction in BW compared to control(for male /female -7%/-5%, -11%/-10%, -21%/-16% in the low, mid and high dose group).
Price (1994)[29] (unpublished) same design of previous study with same dose-regimen and same strain *in utero* and up to 22 months (about 98 weeks) exposure after weaning McFarlane *et al.* (1997)[30]	<u>Parental toxicity</u> F0 male: decreased body weight gain (high-dose) in the first 5 weeks; F0 female: GD20, ↑ both absolute and relative liver weights of the dams in a dose-related manner, statistically significant at 500 mg/kg bw/d for relative liver weight.

[27] Tanaka T, Oishi S, Takahashi O. 1993. Three generation toxicity study of butylated hydroxytoluene administered to mice. *Toxicol Lett. 1993* Mar;66(3):295-304.

[28] Olsen, P., Meyer, O., Bille, N. & Wurtzen, G. (1986). Carcinogenicity study on butylated hydroxytoluene (BHT) in Wistar rats exposed in utero. *Food Chem. Toxicol., 24: 112.*

[29] Price SC, 1994. The role of hepatocellular injury in the chronic toxicity of BHT: Two generation Wistar albino rat study. Robens Institute, U. of Surrey, Guildford, Surrey, U.K. Study No: 1/91/Tx. Final Report No: R193/TOX/0020. Vol. 1-8. Submitted to WHO by Robens Institute. Unpublished.

[30] McFarlane M. Price SC, , Cottrell S., Grasso P., Bremmer JN., Bomhard EM. and Hinton RH. 1997. Hepatic and Associated Response of Rats to Pregnancy, Lactation and Simultaneous Treatment with Butylated Hydroxytoluene. *Food and Chemical Toxicology* 35 (1997) 753-767.

(published) Examination on GDs 19-20, PND21 and offspring 4 and 22 weeks after weaning	<u>Reproduction</u> No effect on reproductive parameters including mating index, gestation index and viability index. F0: Slight decreases in number of pups per litter (low and high-dose), but not dose-related. F1 : Pup body weight from the high-dose group significantly lower than controls at birth (10%), and at days 6 (12%) and 21 (21%) of lactation. Mortality of the pups between day 21 of lactation and culling : 2%, **8%,** 12% and 15%, with increasing dose. Growth retardation observed in the pups claimed to be due to inadequate milk production

The available data do not report effects on fertility. An impairement on pups development is however described consistently among the studies (see also below).

<u>Prenatal developmental toxicity</u>

Studies on reproductive toxicity have been reported in mice, rats, rabbits, chickens and monkeys (WHO, 1996). These are summarized below:

Study **Prenatal development toxicity**	**Observed effects**
RAT <u>Vorhees et al, 1981</u> Sprague-Dawley, at least 13 ♀ Generation study (F0, F1) 0; 0,125; 0,25 or 0,5% in the diet (about 0, 83,167,333 mg/kg bw/d) 14 days before mating, examination of the offpsring up to an age of 90 days	F1 : ↑ postnatal mortality (PNDs 1-30) at 0,25 and 0,5% BHT (significant) ; ↓ BW gain and delayed development before weaning (180° rotation, opening of the eyes, swimming behavior, behavior in the open field), no effects after weaning at 0,5% BHT (significant) ➔ **NOAEL = 83 mg/kg bw/d**
RABBIT <u>FDA 1974</u> Dutch belted at least 12 ♀ GD 16-18 doses 0; 3,2; 14,9; 69,1; 320 mg/kgbw/d	From 3.2 mg/kg bw/d : foetuses:↑ resorptions and ↓number of live fetuses (not strictly related to dose) From 14.9 mg/kg bw/d : ↑maternal mortality (dams : 3/15 died (GDs 9,11, 17) From 69,1 mg/kg bw/d : dams : 3/19 died (GD 11,,13 and 15); 3 abortions (GD 10 and 25), foetuses: considerably ↑ resorptions ; ↓number of live fetuses , markedly ↓ foetal weights *Limits: very variable fertility of the strain used, unusual findings occurring on control group*
MONKEY <u>Allen JR (1976)</u> 6 ♀ rhesus monkeys in a diet containing a mixture of BHT and BHA. Daily intake of 50 mg BHT/kg bw/day and 50 mg BHA/kg bw/day. The monkeys were fed the diet for one year prior to breeding and then for an additional year, including a 165-day gestation period.	No clinical abnormalities were observed in parent or offspring during the period of study. Adult females continued to have normal offspring. Offspring born during the exposure period remained healthy, with the exception of one infant that died from unrelated causes. Home cage observations at the third month of life did not reveal any behavioral abnormalities. **NOAEL = 100 mg/kg bw/day of BHA and BHT combined, the only dose level tested (Efsa panel 2012).**

<u>Postnatal developmental toxicity</u>

Study Postnatal development toxicity	Observed effects
MICE Stokes and Scudder, 1974	Effects on behaviour (sleep; agression) ,learning deficits
RAT Meyer and Hansen, 1980 Wistar rat 40 ♂ / ♀ Generation study (F0, F1) 0, 500 mg/kg bw in semi-synthetic diet starting 13 weeks before mating, examination of the offspring up to the age of 21 days	At 500 mg/kg bw/d, F0: ↓ BW gain (significant) , F1: ↓ bw gain during lactation; delayed development (eruption of teeth, opening of the eyes, and reflexes) during lactation
RAT Eriksson and Siman (1996) pregnant diabetic and normal Sprague-Dawley rats were fed ad libitum either a standard diet or a diet with 1% of BHT (estimated to be equivalent to approximately 500 mg/kg bw/day).	No effect on the rate of resorptions with BHT treatment. No malformations were found in the normal rats treated with BHT. **NOAEL = 500 mg/kg bw/day (the only dose level tested).**

All these studies have been considered of poor reliability (old studies, not statistically powerful studies,…). However, the findings on developmental effects raise concern justifying further testing.

3.2.1.6 Short or subchronic studies

Short-term or subchronic exposure to BHT affects the liver of mice, rats and chickens, also showing histopathological changes in this organ (Takahashi ,1992[31], WHO 1996, Safer and Al-Nughamish, 1999[32], Rao *et al*., 2000)[33]).

In the study of the microsomal enzyme profile in 105 broiler chickens fed *ad libitium* with doses of 130-2080 mg/kg BHT in the diet for 6 weeks, a significant increase ($p>0.01$) in both hepatic microsomal enzymes cytochrome B5 and NADPH-dependent cytochrome P 450 reductase in a dose-dependent manner was observed in the highest BHT group (maxima reached 250% and 162.5%, respectively) (Rao *et al.* in 1999). The authors subsequently reported in 2000 that BHT feeding in chickens (same design as before) caused a marked congestion of the liver and kidney and diffuse enlargement of the liver with rounded borders and rupture with hemorrhage (Rao *et al.*, 2000).

BHT has been shown to increase the relative thyroid and adrenal weight in rats (Johnson and Hewgill, 1961, Gaunt *et al.*, 1965a,). In rat, BHT given orally to male for 7 consecutive days at dose levels of 75 or 450 mg/kg bw/day induced hepatocellular proliferation, an increase in hepatocyte apoptosis, and elevated immunoreactivity for transforming growth factor (TGF)-β1 in the liver during the

[31] Takahashi O, 1992. Haemorrhages due to defective blood coagulation do not occur in mice and guinea-pigs fed butylated hydroxytoluene, but nephrotoxicity is found in mice. *Food and Chemical Toxicology 30*, 89-97.

[32] Safer AM and Al-Nughamish AJ, 1999. Hepatotoxicity induced by the anti-oxidant food additive,butylated hydroxytoluene (BHT), in rats: An electron microscopical study. *Histology and Histopathology* 14, 391-406.

[33] Rao GVS, Parthasarathy KR and Sundararaj A, 2000. Haemorrhagic syndrome in butylated hydroxyl toluene (BHT) toxicity in broiler chicken. Indian Veterinary Journal 77, 117-119.

treatment, and hepatocellular inhibition of mitosis following withdrawal (Furukawa *et al*., 2001[34]). None of the studies available could be used to derive a NOAEL.

3.2.1.7 Genotoxicity

Regarding the genotoxicity, the majority of evidence indicates a lack of potential for BHT to induce point mutations, 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 and that such a mechanism of genotoxicity is generally considered to have a threshold (EFSA Panel 2012).

In summary, BHT reveals no direct genotoxic activities *in vitro* and *in vivo*.

3.2.1.8 Long-term studies and Carcinogenicity

BHT is classified in Carc group 3 by IARC 1986 (for comparison BHA is in group 2B).

- IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, *Some naturally occuring and synthetic food components, furocoumarins and ultraviolet radiation.* IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 40. Lyon : International Agency for Research on Cancer. (1986). [MO-009566] http://www.iarc.fr
- American Conference of Governmental Industrial Hygienists, *2014 TLVs® and BEIs® : threshold limit values for chemical substances and physical agents and biological exposure indices.* Cincinnati (OH) : ACGIH. (2014). Publication 0114. [NO-003164] http://www.acgih.org

Standard carcinogenicity studies in mice and rats suggest that BHT is not carcinogenic (Hiraga 1978[35], NCI 1979[36], Hirose 1981[37], Shirai 1982, WHO, 1996).
The first table below presents mice studies.

MICE Study	Observed effects
Brooks, 1976[38]	Increased incidence of lung neoplasia in treated

[34] Furukawa S, Usuda K, Tamura T, Kubota R, Ikeyama S, Goryo M, Masegi T and Okada K, 2001. Effect of butylated hydroxytoluene on cell population in rat hepatocytes. *Journal of Toxicologic Pathology* 14, 145-150.
[35] Hiraga, K. (1978). Life-span oral toxicity study of 3,5-di- *tert*-hydroxytoluene (BHT) in rats. *Ann. Rep. Tokyo Metropolitan Research Lab. Public Health,* 32: 83.
[36] NCI (1979) National Cancer Institute. Bioassay of butylated hydroxytoluene (BHT) for possible carcinogenicity. *DHEW Report* No. NIH 79-1706. Technical Report Series No. 150.
[37] Hirose, M., Shibata, M., Hagiwara, A., Imaida, K & Ito, N. (1981).Chronic toxicity of butylated hydroxytoluene in Wistar rats.*Food Cosmet. Toxicol.,* 19: 147-151.
[38] Brooks T, Hunt P and *et al*., 1976. Effects of prolonged exposure of mice to butylated hydroxytoluene. Unpublished report from Shell Research, Ltd., Tunstell Lab., Sittingbourne, Kent, UK submitted to the World Health Organization by the authors.

	CFI mice.
Witschi and Cote, 1977[39] Lindenschmidt *et al*., 1986[40]	Increased lung tumors in BALB/c C3H mouse (adenomas of alveolar or Clara cells origin) in a dose dependant manner in females (not dose dependant in males), indications of a tumor-promoting activity of BHT initiated by dimethyl hydrazine (DMH) in BALB/c C3H mouse colon.
Inai *et al.,* 1988[41]	Increased incidence of liver tumors in all dose male groups B6C3F1 mice.
Shearn *et al.,* 2008[42]	Indications of lung tumors BALB/cByJ mouse by inhibition of antioxidant enzymes such as carbonyl reductase (CBR) resulting in accumulation of protein carbonyls in lung cytosol from sustained oxidative stress and explaining lung inflammation in male BALB/cByJ mouse lung throughout a 28-day period of weekly injections of BHT leading to tumour promotion in the two-stage model for pulmonary carcinogenesis.

Increased rates of hepatocellular tumors were observed in Wistar rats in two reports (Olsen *et al.*, 1986, Price *et al*. 1994).

Wistar RAT Study	**Observed effects**
Olsen *et al.,*1986	Dose-related increase in hepatocellular carcinomas in males; increase in hepatocellular adenomas in males and females. Tumours included thyroid, pancreas, ovary, uterus, thymus, reticulo-endothelial system, and mammary gland, but incidence was claimed to be not statistically significantly different from that in controls respectively. NOAEL for non-neoplastic effects = 25 mg/kg bw/day
Price, 1994	Increase in hepatocellular content and distribution of CYP P450 2B Induction GGT activity; increase by 30-60% in total cytochrome P450 content in the high-dose

[39] Witschi H and Cote MG, 1977. Inhibition of Butylated Hydroxytoluene-Induced Mouse Lung Cell Division by Oxygen: Time-Effect and Dose-Effect Relationships. *Chemico Biological Interactions 19*, 279-289.

[40] Lindenschmidt RC, Tryka AF, Goad ME and Witschi HP, 1986. The effects of dietary butylated hydroxytoluene on liver and colon tumor development in mice. *Toxicology 38*, 151-160.

[41] Inai, K., Kobuke, T., Nambu, S., Takemoto, T., Kou, E., Nishina, H., Fujihara, M., Yonehara, S., Suehiro, S. & Tsuya, T. (1988). Hepatocellular tumorigenicity of butylated hydroxytoluene administered orally to $B6C3F_1$ mice. *Jpn. J. Cancer Res.,* 79(1): 49-58.

[42] Shearn CT, Fritz KS, Meier BW, Kirichenko OV, Thompson JA. 2008. Carbonyl reductase inactivation may contribute to mouse lung tumor promotion by electrophilic metabolites of butylated hydroxytoluene: protein alkylation in vivo and in vitro. Chem Res Toxicol. 2008 Aug;21(8):1631-41. doi: 10.1021/tx800162p. Epub 2008 Jul 3.

	animals, dose-related increase in epoxide hydrolase, glutathione-S-transferase and pentoxyresorufin-O-dependentylase (PROD) activities, starting at 21 days of age, statistically significant in the mid- and high-dose groups; Thyroid: decrease follicular size, absence or decrease colloid, irregularities in the follicular outline, hyperaemia and increase in number of follicular cells 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). No change in serum thyroxin levels.
Maeura and Williams, 1984[43]	Significant increased number of foci, the area occupied by GGT-positive preneoplastic and neoplastic lesions and the neoplasm incidence at high dose level of BHT initiated by N-2-fluorenylacetamide (FAA) F344 rat liver.
Ito *et al.,* 1986[44]	Some indications of a tumor-promoting activity of BHT in F344 rat urinary bladder initiated by N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) (increase incidence in papilloma: BBN/1% BHT 17 (70.8) (P<0.01) vs BBN/2% BHA 22 (88) (P<0.001) and incidence in cancer: BBN/1% BHT 13 (54.2) P<0.05 vs BBN/2% BHA 19 (76) (P<0.001) and also indications of promoting effects of BHT in thyroid (adenoma) initiated with N-methyl-N-nitrosurea (MNU) : MNU/5.0% BHT 11 (64.7) p<0.05) in male F344 rats.

The results of the studies showed that BHT cause hepatocellular tumors in rats with induction of liver enzymes. This mechanism can be considered relevant for humans. Therefore these hepatocellular tumors will be considered for a classification. Some effects were also observed on thyroid (see below section 3.2.1.11). In depth analysis of the exisiting data on carcinogenesis will be conducted while evaluating the substance to decide if a classification is warranted.

3.2.1.9 Mechanistic studies

Various mechanisms explaining lung and liver tumors are reported in certain strains of mice and rats :

- inhibition of gap functional intercellular communication (Guan *et al*., 1995[45], Yamasaki, 1996, Trosko *et al.*, 1990[46]);

[43] Maeura Y and Williams GM, 1984. Enhancing Effect Of Butylated Hydroxytoluene On The Development Of Liver Altered Foci And Neoplasms Induced By N-2-Fluorenylacetamide In Rats. *Food and Chemical Toxicology 22*, 191-198.

[44] Ito N., Hirose M., Fukushima S., Tsuda H., Shirai T. and Tatematsu M.. Studies on antioxidants : their carcinogenic and modifying effects on chemical carcinogenesis. *Toxic.* Vol. 24, No. 10/11, pp. 1071-1082, 1986

- increase in mitochondrial permeability (Sokolove *et al.*, 1996[47]);
- epigenetic changes due to induction of DNA methyl transferases (Vanyushin *et al.*, 1998);
- oxidative stress:

Vanuyshin *et al.* in 1998[48] reported a strong increase in nuclear DNA (cytosine-5)-methyl transferase activity in the liver, kidneys, heart, spleen, brain and lungs of male rats within a few hours following a single intraperitoneal injection of 60 mg BHT kg bw; induction of oxidase stress by the quinone methide metabolite in heart rat (Faine *et al.*, 2006), in mouse lung (Kupfer *et al.*, 2002[49]); inhibition of antioxidant enzymes such as carbonyl reductase (CBR) resulting in accumulation of protein carbonyls in lung cytosol from sustained oxidative stress and explaining lung inflammation in male BALB/cByJ mouse lung throughout a 28-day period of weekly injections of BHT leading to tumor promotion in two-stage model for pulmonary carcinogenesis (Shearn *et al.*, 2008).

In vitro non-tumorigenic immortalized lung epithelial cell lines C10 and E10 and their corresponding tumorigenic siblings A5 and E9, respectively showed that BHT-derived quinone methides may exert their promoting effects by inducing oxidative stress; such stress is better tolerated by tumorigenic cells, which have higher levels of antioxidant enzyme. Normal cells are destroyed more readily which allows neoplastic cells to expand their proliferation.

The mechanism of DNA damage by the BHT metabolites (BHT quinone and BHT-COOH) was investigated in an *in vitro* study in cellular and acellular systems. Based on the results obtained, the authors suggested that following metabolic conversion BHT may induce oxidative damage to DNA through two different pathways, i.e. the oxidation by BHT-OOH in presence of transition metals, and the intracellular generation of H_2O_2 by BHT-quinone (Oikawa *et al*., 1998[50]).

Later a range of studies have been conducted which aim to illuminate basic biochemical and molecular biological effects of BHT on various organs, mainly the liver. BHT was studied in the concentration range of 0-50 µM *in vitro* in opsonised zymosan stimulated neutrophils obtained from New Zealand White Rabbits. BHT showed concentration-dependent cytotoxicity, interaction with neutrophil membranes and reactive oxygen species (ROS) scavenger activity. It was pointed out by the study authors that BHT exerted a cytotoxic effect probably mediated

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[45] Guan X, Hardenbrook J, Fernstrom MJ, Chaudhuri R, Malkinson AM, Ruch RJ. 1995. Down-regulation by butylated hydroxytoluene of the number and function of gap junctions in epithelial cell lines derived from mouse lung and rat liver. Carcinogenesis. 1995 Oct;16(10):2575-82.

[46] Trosko JE, Chang CC, Madhukar BV, Klaunig JE. 1990. Chemical, oncogene and growth factor inhibition gap junctional intercellular communication: an integrative hypothesis of carcinogenesis. Pathobiology. 1990;58(5):265-78.

[47] Sokolove PM, Haley LM. Butylated hydroxytoluene and inorganic phosphate plus Ca2+ increase mitochondrial permeability via mutually exclusive mechanisms. J Bioenerg Biomembr. 1996 Apr;28(2):199-206.

[48] Vanyushin BF, Lopatina NG, Wise CK, Fullerton FR and Poirier LA, 1998. Butylated hydroxytoluene modulates DNA methylation in rats. European Journal of Biochemistry 256, 518-527.

[49] Kupfer R, Dwyer-Nield LD, Malkinson AM, Thompson JA. 2002. Lung toxicity and tumor promotion by hydroxylated derivatives of 2,6-di-tert-butyl-4-methylphenol (BHT) and 2-tert-butyl-4-methyl-6-iso-propylphenol: correlation with quinone methide reactivity. Chem Res Toxicol. 2002 Aug;15(8):1106-12.

[50] Oikawa S, Nishino K, Inoue S, Mizutani T and Kawanishi S, 1998. Oxidative DNA damage and apoptosis induced by metabolites of butylated hydroxytoluene. *Biochemical Pharmacology* 56, 361-370.

by an interaction with neutrophile membranes and a scavenging of reactive oxygen species which could produce reactive intermediates (Kabeya *et al*., 2008[51]; Saito *et al*., 2003[52]).
BHT as a pro-oxidant at high concentrations, indirectly inhibits antioxidant defenses through the depletion of non protein thiols and by covalent modifications of protective enzymes (Sun *et al*., 2003[53]).

3.2.1.10 Epidemiological studies of stomach cancer

The association between dietary intake of BHT and stomach cancer risk was investigated in the Netherlands Cohort Study (NLCS) that started in 1986 among 120 852 men and women aged 55- 69 years. Mean intake of BHT among sub-cohort members was 0.351 mg/day. No significant association with stomach cancer risk was found in this study, for normal dietary intake of low levels of BHT (Botterweck *et al*., 2000). It is also noted that no association between stomach cancer risk and mean BHA (105 µg/day) was reported in this cohort study.

Conclusion on carcinogenicity

The International Agency for Research on Cancer (IARC) evaluated BHT (1987)[54] and classified it in group 3, since no evaluation could be made of the carcinogenicity of BHT to humans, and there was limited evidence for the carcinogenicity in experimental animals. Williams *et al*. (1999) argued that BHT is not genotoxic or carcinogenic; they particularly argued that the carcinogenicity in rats found in the study by Olsen *et al*. (1986) has not been confirmed in other studies with rats, and that the effects observed may be attributable to study conditions and not to the administration of BHT. They also point out that a more recent study (Takagi *et al*., 1994[55]) dosing Wistar rats for up to 18 months with 0.1% 2,2'-methylenebis (4-methyl-6-tertbutylphenol), an antioxidant which is essentially two molecules of BHT and has all the attributes of BHT, did not result in a carcinogenic response.

Increased incidence of hepatocellular tumors in Wistar rats and B6C3F1 mice was probably induced under special experimental conditions (rat; study period up to

[51] Kabeya LM, Kanashiro A, Azzolini AE, Santos AC and Lucisano-Valim YM, 2008. Antioxidant activity and cytotoxicity as mediators of the neutrophil chemiluminescence inhibition by butylated hydroxytoluene. *Pharmazie* 63, 67-70.
[52] Saito M, Sakagami H and Fujisawa S, 2003. Cytotoxicity and apoptosis induction by butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). *Anticancer Research* 23, 4693-4701.
[53] Sun Y, Dwyer-Nield LD, Malkinson AM, Zhang YL, Thompson JA. 2003. Responses of tumorigenic and non-tumorigenic mouse lung epithelial cell lines to electrophilic metabolites of the tumor promoter butylated hydroxytoluene. Chem Biol Interact. 2003 Mar 6;145(1):41-51.

[54] IARC, 1987. Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation Summary of Data Reported and Evaluation. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 40. Available from: http://monographs.iarc.fr/ENG/Monographs/vol40/volume40.pdf.
[55] Takagi A, Takada K, Sai K, Ochiai T, Matsumoto K, Sekita K, Momma J, Aida Y, Saitoh M, Naitoh K *et al*., 1994. Acute, subchronic and chronic toxicity studies of a synthetic antioxidant, 2,2'- methylenebis (4-methyl-6-tert-butylphenol) in rats. *Journal of Toxicological Sciences 19*, 77-89.

144 weeks)(Olsen *et al*. 1986) or at very high doses (mouse: 3480 mg/kg bw and day for 2 years)(Inai *et al*. 1988). BHT revealed a tumor-promoting effect in some 2-stage initiation-promotion experiments. Investigations into the inhibition of intercellular communication *in vitro* and into the stimulation of cell proliferation *in vitro* and *in vivo* suggest that the hepatocarcinogenic effects of BHT that is observed under special conditions is due to proliferation or tumor-promoting properties. The tumor-promoting activity of BHT initiated by a genotoxicant was observed in various organs:

- in mouse lung : increased adenomas of alveolars or Clara cells origin in BALB/c C3H mouse,
- in mouse colon: no definitive answer regarding the increase of colon tumors after dimethyl hydrazine (DMH) treatment but not after N-methyl-N-nitrosurea (MNU) (Lindenschmidt *et al*., 1986),
- in rat urinary bladder: increases in papilloma tumors after N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) (Ito *et al.,* 1986)).

BHT has a tumoral effect on liver and lung and exert tumour-promoting effects on other organs in some animal models (depending on treatment sequences and initiator, and species). **In depth analysis of the exisiting data on carcinogenesis will be conducted while evaluating the substance to decide if a classification is warranted.**

3.2.1.11 Endocrine disruptor characteristics for human

3.2.1.11.1 Effects on adrenals

BHT has been shown to increase the relative adrenal weight in rats. In the report of Johnson & Hewgill[56] in 1961, 6 weanling Norway hooded rats (3 per sex) received in diet a 20% lard supplement and BHT at levels of 0, 0.1, 0.2, 0.3, 0.4 or 0.5% (equivalent to 0, 150, 300, 450, 600 or 750 mg/kg bw/d) for 6 weeks. BHT reduced the growth rate, especially in males, the effect becoming significant at 0.3% BHT (sex difference in the effect of BHT noted by Brown *et al.*, 1959[57]). BHT increased the ratio of left adrenal weight to body weight in male rats but had no consistent effect in females. There were no histological changes in the adrenal attributable to treatment.

In the report by Gaunt *at al.* in 1965a[58]_48 weanling Carworth SPF rats (24/sex) received 0 or 0.1% (equivalent to 0 or 150 mg/kg bw/d) BHT in diet for periods of up to 16 weeks. Measurements of growth rate, food consumption, weight and micropathological examination of organs at autopsy revealed no difference between treated and control groups. However, increase in relative liver weight and in the weight of the adrenals was observed without histopathological evidence of damage. Observations on the adrenal in these studies must be interpreted with caution because the weight was standardized on the weight of the animal with a small difference (0.51 mg / g for the controls vs 0.56 mg / g for animals exposed to BHT). In the report by Price *et al*. 1994 described above in reprotoxicity section, no effects on the adrenal was observed. Histopathology of the adrenal was conducted starting at- 11 months post-weaning. It is to be noted that in a nearly identical, but invalidated study in Wistar rats conducted in the same laboratory (*Robens Institute, Price 1994*) cytomegaly of cells of the zona fasciculata was observed in the mid- and high-dose groups at weaning and at 4 weeks post-weaning, but not at subsequent time points. **We therefore consider that the effects of BHT on the weight on adrenals in different strains of rats are of no relevant significance.**

3.2.1.11.2 Effects on thyroid

There are some studies where effects on thyroid and/or effects linked with a probable effects linked to thyroid were evaluated.

Both 2-generation studies (Olsen *et al*., in 1986; Price *et al*., 1994) indicated effects on the thyroid. These studies were described above (in section on reprotoxicity) and analyzed by the Efsa panel in 2012. In the report by Olsen *et al.* in 1986, thyroïd tumors not statistically significantly different from that in

[56] Johnson A.R. and Hewgill F.R., 1961. The effect of the antioxidants, butylated hydroxyl anisole, butylated hydroxyl toluene and propyl gallate on growth, liver and serum lipids ans serum sodium levels of the rat. Austral. J. exp. Biol. (1961), 39, pp. 353-360.

[57] Brown W.D., Johnson A.R., and O'Halloran M.W; 1959. The effect of the level of dietary fat on the toxicity of phenolic antioxidants. Aust. J. exp. Biol. (1959), 37, pp.533-548.

[58] Gaunt IF, Feuer G., Fairweather FA and Gilbert.D. 1965. Liver Response Tests. IV. Application to Short-term Feeding Studies with Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA). Fd Cosmef. Toxicol. Vol. 3, pp. 43343.(1965).

controls were reported. In the study by Price *et al*. (1994), evidence of thyroid hyper-activity, characterized by reduction of follicular size, absence or reduction of colloid, irregularities in the follicular outline, hyperaemia and 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 in the -low and –mid dose group from controls, T4 is increased not significantly in the high dose group which seems coherent with an hyperactivity of the thyroid observed only in the high-dose-group. In parallel, some effects on hepatic enzymes (CYP 450 cytochrome) induction and increase in dose-related manner in epoxide hydrolase, glutathione-S-transferase and pentoxyresorufin-O-depentylase (PROD) activities, starting at 21 days of age, statistically significant in the mid- and high-dose groups were also observed. There is a real need to clarify the link between the disruption in thyroid-pituitary fonction and the induction of hepatic enzyme.

An interesting publication by Sondergaard and Olsen 1982 studied the thyroid action, other studies (Johnson *et al.* 1961 and Gaunt *et al.*1965) simply reported the effects on the thyroid without checking data or without further details.

In the 90 day exposure study reported by Sondergaard and Olsen (1982) and performed in Wistar rats (10-30 males) receiving 0, 500 or 5000 ppm BHT in a semi-synthetic diet (estimated to be equivalent to approximately 0, 25 or 250 mg/kg bw/day) for 8, 26, 62 or or 90 days, the uptake of Iode ^{125}I by the thyroid was determined. The presence of BHT at the dose level of 250 mg/kg bw/d in the diet resulted in a significant increase in the uptake of Iode ^{125}I at all time periods studied. When rats were fed BHT at 250 mg/kg bw/d in semi-synthetic diets containing varying amounts of iodine (0.12, 0.15 or 0.3 mg iodine/kg feed) for 30 days, there was a significant ($p<0.001$) increase in absolute thyroid weight in BHT treated animals when compared to controls. BHT did not change levels of T3 and T4 in the blood. The biological half-life of thyroxine was increased ($p< 0.01$) after 13 days on a BHT diet but returned to normal after 75 days. From 25 mg BHT/kg bw/day, a significant increase in relative thyroid weight was observed. Electron microscopy of the thyroid glands of rats exposed to 250 mg BHT/kg bw/day for 28 days showed an increase in the number of follicle cells, a significant increase in absorption of iodine, a significant increase in relative liver weight were also observed at this dose level (WHO, 1996; Sondergaard and Olsen, 1982[59]).

As described above some effects on hepatic enzymes (CYP 450 cytochrome) induction and consequent thyroid hyperactivity in the mid- and high-dose groups in rat were observed in the unpublished study by Price *et al*, 1994. Although rodents and humans share a common physiology in regard to the thyroid-pituitary feedback system, a number of factors contribute to the greater sensitivity of the rat to long-term perturbation of the pituitary thyroid axis which predisposes it to a higher incidence of proliferative lesions in response to chronic TSH stimulation than human thyroid.

Both humans and rodents have non specific low affinity protein carriers of thyroid hormone (e.g., albumin). However, in humans, other primates, and dogs there is a high affinity binding protein, thyroxine-binding globulin (TBG), which has a 1000-fold greater binding affinity than non specific low affinity protein carriers and which binds T4 (and T3 to a lesser degree); this protein is not present in

[59] Søndergaard D, Olsen P.1982. The effect of butylated hydroxytoluene (BHT) on the rat thyroid. *Toxicol Lett.* 1982 Feb;10(2-3):239-44.

rodents, birds, amphibians and fish: therefore, circulating thyroïd hormons is lower in humans than in rodent.
Although qualitatively the rat is an indicator of a potential human thyroid cancer hazard, humans appear to be quantitatively less sensitive than rodents to developing cancer from perturbations in thyroid-pituitary status. Given that the rodent is a sensitive model for measuring the carcinogenic influences of TSH and that humans appear to be less responsive, effects on rodents would represent a conservative indicator of potential risk for humans. Rodent cancer studies typically include doses that lead to toxicity, including perturbation in thyroid-pituitary function over a lifetime. The relevance of the experimental conditions to anticipated human exposure scenarios (i.e., dose, frequency and time) should be considered. In addition, chemically-induced effects that are produced by short-term disruption in thyroid-pituitary function appear to be reversible, when the stimulus is removed.

In summary the study by Sondergaard and Olsen 1982, thyroid physiology seems actually affected by exposure to the BHT from 5000 ppm (250 mg/kg bw/day) in the diet. In particular, a significant increase of iodine capture by the thyroid is observed and associated with thyroid hyperplasia. The authors Sondergaard and Olsen propose a hepatic primary mechanism: hepatic enzyme induction and increased catabolism of thyroid hormones lead to a compensatory cascade involving an acceleration of iodine cycle in connection with the increased needs to maintain circulating levels. But this scheme of mechanism is not so evident. Several pathophysiological links to this scheme are not documented in any the studies cited above or in the study by Sondergaard *and Olsen* 1982.

It was proved that fipronil-induced disruption of thyroid function in rats is mediated by increased total and free thyroxine clearance concomitantly to increased activity of hepatic enzymes (Leghait *et al.*, 2009[60]). However, this link has not be proven with BHT. There is no direct evidence that the toxic mechanisms on the thyroid are due to increased hepatic metabolism of the thyroid hormones (TH). If such mechanism was true, thyroxine (T4) biological half-life would be expected to be shorter in BHT-treated animals whereas T4 half-life is increased in response to BHT treatment. Moreover the method is based on the measurement of total radioactivity following an injection of radiolabeled T4 including ^{125}I-T4 but also all other iodinated metabolites or even free ^{125}I that arise from T4 metabolism, it can be hypothesized that the increased in "the so called T4 half-life" might be explained at least in part to an accelerated iodine recycling and recirculation. Other mechanisms explaining the effects on thyroid could be proposed such an increase in iodin incorporation rate by the thyroid due to increased expression of the iodine transporter NIS that can be triggered by increased TSH secretion or due to increased TSH leading to the development of thyroid hyperplasia.

In the present case, there is no information about a possible induction to hepatic enzyme systems key catabolism thyroid (deiodinases, UGT, sulfotransferases). There are no data on circulating concentrations of pituitary TSH which is the initiator of the compensatory cascade to maintain thyroid homeostasis and the establishment of hyperplasia related to prolonged hyperstimulation of the gland.

[60] Leghait J., Gayrad V., Picard-hagen N., Camp M., Perdu E., Toutain PL., Viguié C. Fipronil-induced disruption of thyroid function in rats is mediated by increased total and free thyroxine clearance concomitantly to increased activity of hepatic enzymes. Toxicology 255 (2009) 38-44.

In conclusion,, from the existing data, thyroïd is affected in rats. There is a little information showing that this might be secondary to the hepatic metabolic activation. However, key parameters are missing to validate this MoA. There is no information regarding the induction of pathways specifically involved in TH catabolism, the data are inconclusive regarding T4 kinetic (AUC, clearance, terminal half-life, T_{max}, C_{max}) and there is no quantitative data regarding circulating TSH that proves that TSH secretion is modified. Therefore, further data should be gathered before being able to conclude on BHT effect on thyroïd.

BHT belongs to alkylphenols which showed estrogenic activity (Hughes *et al*, 2000[61]). Industrial alkylphenols in the environment may act as "xenoestrogens" and disrupt testicular development and decrease male fertility. The possible targets are testicular Sertoli cells, which nurture the developing sperm cells and SERCA 2 and 3 Ca(2+) pumps relatively abundant in rat testis microsomal membranes, in Sertoli, myoid, and TM4 cells (a Sertoli cell line). The study by Hughes *et al*. reported a number of estrogenic alkylphenols such as nonylphenol, octylphenol, bisphenol A, and butylated hydroxytoluene which inhibit testicular Ca(2+) ATPase in the low micromolar concentration range using immunohistochemical method. They mobilize intracellular Ca(2+) in intact TM4 cells in a manner consistent with the inhibition of ER Ca(2+) pumps. They dramatically decrease the viability of TM4 cells, an effect that is reversed by either a caspase inhibitor or by BAPTA, and is therefore consistent with Ca(2+)-dependent cell death *via* apoptosis. In conclusion, alkylphenols may disrupt testicular development by inhibiting ER Ca(2+) pumps, thus disturbing testicular Ca(2+) homeostasis.

In vitro estrogenic and androgenic activities of BHT were evaluated in two studies.

The study of Wada *et a*l. in 2004[62] tested the hypothesis that commercial composites, which contain various monomers and additives, exhibit estrogenic activity *in vitro*. The estrogenic activities of eluates obtained from 24 composites and 18 chemicals identified from the composites tested were examined with the use of the reporter gene assay (luciferase activity). The assay is of better specificity and sensitivity than the other methods for screening estrogenic activity. Among the 24 composites in mixture, 6 products were estrogenic, and among the 18 constituents, 1 photostabilizer, 2-hydroxy-4-methoxy-benzophenone (HMBP), 1 photoinitiator, 2,2-dimethoxy-2-phenyl-acetophenone (DMPA), and 1 inhibitor, 2,6-di-tert-butyl-p-cresol (BHT) had significant estrogenic activity at 50 µmol/L.

In the report by Schrader, TJ et GM Cooke in 2000[63] the androgenic effects of organochlorine food contaminants (0, 0.1, 1.0, and 10.0 microM) on luciferase activity in PC-3 LUCAR+ cells were determined after exposure to the chemical for 18 h in the presence and absence of 5alpha-Dihydrotestosterone (DHT) (50 pM). BHT at 10 µM completely antagonized the activation of DHT (50 pM). BHT

[61] Hughes and al 2000. Estrogenic alkylphenols induce cell death by inhibiting testis endoplasmic reticulum Ca(2+) pumps, Biochem Biophys Res Commun. 2000 Nov 2;277(3):568-74.

[62] Wada, H. *et al*., 2004. «In vitro estrogenicity of resin composites», Journal of Dental Research 83, no. 3 (mars 2004) : 222-6.

[63] Schrader, TJ et GM Cooke, 2000. «Examination of selected food additives and organochlorine food contaminants for androgenic activity in vitro», Toxicological Sciences 53, no. 2 (février 2000) : 278-88. http://davidsuzuki.org/issues/health/science/toxics/chemicals-in-your-cosmetics---bha-and-bhti

completely inhibited the activation by DHT without having deleterious effects on cell viability. BHT could act as an androgen antagonist.

The only available data are these two *in vitro* studies showing that BHT has an effect on steroidogenesis and anti-androgenesis. **However it is interesting to note that the 3D-structure of BHT seems incompatible with such activation. This pathway needs to be further studied.**

3.2.1.12 Immunological effects

Some effects of BHT were reported on the immune system.

In mice some effects of immunotoxicity were reported in a study by Kim *et al.* in 1996[64] on the suppression of *in vitro*-T cell-dependent humoral immunity by antioxidants using spleen cells from SPF BALB/c mice. Using a modified suspension hemolytic assay, BHT was dissolved in 1, 10, and 100 mM DMSO and diluted in medium for final concentrations of 0.1, 1, and 10 μM. BHT suppressed the T cell–dependent B-cell response, but not the T cell–independent and polyclonal B-cell responses. Therefore BHT did not directly suppress B cells; rather, humoral immunity was inhibited by suppression of regulation of T cells or by the action of macrophages on B cells. In an *in vivo* study by Harman, Heidrick, and Eddy in 1977[65],female C3HeB/FeJ mice received 0.25% BHT in diet, then after approximately 30 days an intraperitoneal injection with 0.1 cc of a 20% suspension of sheep red blood cells (SRBCs). Mice were killed 5 days after the injection and spleen assayed for the cells secreting anti-SRBC antibody. There was evidence that BHT enhanced significantly humoral immune response compared to control groups.

3.2.1.13 Overall conclusions for human hazards

Despite the wide use of BHT as an ingredient, only very few cases of allergic reactions in humans after dermal exposure or by oral intake have been described (OECD 2002).

The genotoxicity studies on BHT were limited but the majority of evidence indicates a lack of potential of BHT to induce point mutations, chromosomal aberrations, or to interact with, or damage, DNA. It was recognised that positive genotoxicity results obtained with BHT *in vitro* may be due to pro-oxidative chemistry giving rise to formation of quinones and reactive oxygen species. Such a mechanism has to be evaluated further before concluding on its relevance in terms of hazard identification.

Based on two 2-generation studies and long-term studies including carcinogenicity studies, a NOAEL of 25 mg/kg bw/day was established by EFSA. At higher levels increased incidence of hepatocellular carcinoma occurred in male

[64] Kim HM, Han SB, Chang WI, Hyun BH, Oh GT, Ahn CJ, Cha YN. Selective suppression of in vitro T-dependent humoral immunity by synthetic food additive antioxidants. J Toxicol Sci. 1996 Feb;21(1):41-5.

[65] Harman D, Heidrick ML, Eddy DE.Free radical theory of aging: effect of free-radical-reaction inhibitors on the immune response. J Am Geriatr Soc. 1977 Sep;25(9):400-7.

rats and effects on litter size, sex ratio and pup body weight gain were noted. BHT has promoting effects in some animal models at high doses.

Overall the hazard evaluation made in the OECD SIDS, in the REACH registration dossier and in the recent EFSA evaluation can be considered comparable. It seems a bit unclear, however, whether the carcinogenicity data may justify an harmonised classification as Carc 2 (CLP).

The substance has some effects on the adrenals, but they are not of relevant significance. 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.

BHT has postnatal developmental effects in rats and mice (behavior disturbances, delayed development).

BHT belongs to alkylphenols compounds and industrial alkylphenols in the environment may act as "xenoestrogens" which disrupt testicular development and decrease male fertility. Some limited *in vitro* studies showed that BHT could act as an estrogen and an anti-androgen although the 3D-structure of BHT seems incompatible with such activation.

Another endpoint to consider is that BHT can transfer in placenta and can be found in rat milk (Olsen *et al*, 1986, McFarlane, 1997) that implies to be careful on vulnerable populations (pregnant women and young children) in which even minimal modification of thyroid homeostasis could be harmful in particular for future neuro-cognitive development. This is even more important given that the very few multigenerational studies conducted in rat and mice indicate the occurrence of impaired growth and neurocognitive development (Vorhees *et al*, 1981, Tanaka *et al* 1993, Stokes and Scudder 1974). Although this type of alterations can be related to gestational and/or neonatal thyroid disruption, no data is provided for establishing the link in the case of BHT.

Although there is converging pieces of evidence suggesting that BHT might act on thyroid homeostatis through increased TH hepatic catabolism, in the current knowledge, there is no direct proof that this mechanism holds true. As this mechanism is considered as controversial to human, in terms of its physiological consequences, it seems important to:

1) validate this hypothetical mechanism in the rat using proper pharmacokinetic investigations allowing to avoid any confusion between thyroid hormone, their metabolites and free iodine kinetics. Biochemical investigations on systems (enzymes and transporters) devoted to TH catabolism in the liver should also be conducted.

2) check for the occurrence of such mechanisms in animal species and/or *in vitro* system relevant to human in terms of TH specific binding protein, providing that these species will exhibit the same metabolic pattern for BHT in human.

3) check if pregnancy can be a factor of increased sensitivity to this type of mechanisms due to the high level of solicitation of the maternal organisms to insure the fetal needs in terms of energetic metabolism and thyroid hormones.

In conclusion even if the studies are warnings about the potential effect of BHT to disrupt the hormonal system, the level of information available is limited. Evaluations are based on old studies, not always available, of poor reliability, with limited reports, not statistically powerful, ...). The other effects of BHT (hypersensitivity to the worker (skin contact), reproductive toxicity, neurotoxicity sensitive populations) have to be examined further. Therefore, BHT is proposed for evaluation under REACH.

3.2.1.14 DNELs derivation

For information the DNEL values derived by the registrants, based on a starting point of 10 mg/kg bw/d, which is considered the no effect level for non-adverse enzyme induction in the liver are indicated in annex 2.

Anses will propose DNEL values while evaluating the substance.

3.2.2 E-fate and ecotoxicity

BHT was reviewed under the OECD Cooperative Chemicals Assessment Programme, and as no additional information was provided in the CSRs compared to the OECD dossier, most of the conclusions were proposed in accordance with the SIDS (SIAM 14, 26-28 March 2002).

BHT has a melting point of ca. 70 °C, water solubility in the range from 0.6 to 1.1 mg/L (20-25 °C), a density of 1.03 g/cm³, and a vapor pressure of 1.1 Pa (20 °C). The measured log Kow is determined to be 5.1.

In air BHT is indirectly photodegradable by hydroxyl radicals with $t_{1/2}$ = 7.0 hours (Atkinson method). No hydrolysis study is proposed considering that BHT structure contains functional groups with weak potential for dissociation. BHT is not readily biodegradable in water according to a modified MITI-I test (4.5 % degradation after 28 days). Nevertheless, taking into account its chemical structure and its function as antioxidant, BHT is relatively unstable in the aquatic compartment under environmental conditions as demonstrated by different studies. Extent and products of decomposition are dependent on several factors like irradiation, pH, temperature, moisture, presence of soil and soil microorganisms, and oxygen content. In aqueous solution BHT is decomposed in natural sunlight with irradiation (ca. 75 %) and without (ca. 40 %), forming different, partly unidentified reaction products. BHT is also not stable in soil. Within one day of incubation 63-82 % of BHT was decomposed in non-sterilized and 25-35 % in sterilized soils. A mineralization up to 30 % was observed under non-sterilized conditions after 24 days. In soil, more than 10 degradation products, identified an non-identified, were found. No information is available to define if metabolites are major or minor.

The soil adsorption is extrapolated from the Kow value with the computer program KOCWIN (US-EPA; EPIWIN software) and the Sabljic molecular connectivity (MCI) method. The estimated values range from 8183 to 23030 L/kg. Depending on the exposure pathways, the compartments air, hydrosphere and soil can be environmental target compartments for this substance and its metabolites.

A wide range of bioconcentration factors (BCF) was found in different experiments. Bioconcentration factors (BCF) in the range of 220-2800 have been determined for fish after 56 days (MITI studies). However this study exhibits methodological deficiencies concerning number of exposed test organisms and analytical test procedure. Low BCF values were also reported in data published on the concentration of BHT in human fat tissue, but they cannot be compared with BCF values determined for aquatic species. The substance has been evaluated by Sweden for the EU PBT Working Group (PBT Summary Sheet No. 121, 2004) and more details on the MITI studies were provided to clarify the bioaccumulation issue. The conclusion of the rapporteur was the following: despite the fact that

these studies have a lot of weaknesses (e.g. use of dispersants, not optimal sampling regime, large variations, etc.) they are judged to give sufficient evidence to conclude that BHT does not meet the B-criterion.

For the ecotoxicity of BHT on aquatic species, reliable experimental results from tests with fish, daphnia, and algae are available. Only those effect values are considered for the assessment that did not exceed the low water solubility of BHT (0.6 - 1.1 mg/L) and were based on measured concentrations. According to OECD SIDS, the lowest reliable acute toxicity values were: fish (*Brachydanio rerio*): 96h $LC_0 \geq 0.57$ mg/L; invertebrates (*Daphnia magna*): 48h $EC_0 \geq 0.17$ mg/L; algae (*Scenedesmus subspicatus*): 72h $ErC_8 = 0.4$ mg/L. This value can be used as a NOEC. In a 21 days reproduction test with *Daphnia magna* a NOEC = 0.07 mg/L was determined. Using an assessment factor of 50, a PNECaqua = 0.0014 mg/L is derived from this long term NOEC.

3.2.3 Endocrine disruptor characteristic of BHT for the environment

Literature data do not show any signals on potential endocrine effects on the environmental organisms.

Experts from ED group agree with France's conclusions based on the current avalilable data (following ED expert group discussions taking place in Helsinki at ECHA the 21-22th of October 2015). The amphibian test (eg.LAGDA) would probably be appropriate to answer environmental challenges and to confirm a possible effect on thyroid in mammals. LAGDA assay (highlighting adverse effects) is prefered to a test on fish as BHT has an activity on thyroid and it has low androgenic or estrogenic activity. HSE UK agency, Danish EPA and AGES Austrian agency confirm that the long-term fish test according to OECD 234 is not relevant. Danish EPA and AGES confirm that LAGDA assay is the most relevant test.

3.3 PBT assessment

BHT is not readily biodegradable according to the results of standard tests. There are some indications of degradation from other studies and from related substances, and the substance would be expected to react with oxidants in the environment in view of its intended function; as a result, inherent degradability in the environment can be assumed. In 2004, the EU PBT Working Group concluded that BHT meets the P/vP criterion based on available data. In fact the environmentally relevant rate of degradation is not known. Degradation rate in sediment may deviate considerably from the degradation rate in water due to a different oxygen regime. The substance has been observed to degrade in soil but the rate of degradation cannot be judged on the basis of the data. Further testing on degradation would be needed especially for soil and sediment to determine environmentally relevant rates of degradation (PBT Summary Sheet No. 121, 2004).

A wide range of bioconcentration factors (BCF) was found in different experiments. Bioconcentration factors (BCF) in the range of 220-2800 have been determined for fish. Nevertheless, an in-depth assessment of the studies by the rapporteur (SE) of the EU PBT Working Group allowed concluding that BHT does not meet the B criterion (PBT Summary Sheet No. 121, 2004).

Based on the lowest toxicity endpoint from the 21 days reproduction test with Daphnia magna (NOEC = 0.07 mg/L), BHT does not meet the T criterion.

To conclude, BHT should not be considered as a PBT substance.

4 INFORMATION ON (AGGREGATED) TONNAGE AND USES[66]

4.1 Tonnage and registration status

Table: Tonnage and registration status

From ECHA dissemination site		
☒ Full registration(s) (Art. 10)	☐ Intermediate registration(s) (Art. 17 and/or 18)	
Tonnage band (as per dissemination site)		
☐ 1 – 10 tpa	☐ 10 – 100 tpa	☐ 100 – 1000 tpa
☒ 1000 – 10,000 tpa	☒ 10,000 – 100,000 tpa	☐ 100,000 – 1,000,000 tpa
☐ 1,000,000 – 10,000,000 tpa	☐ 10,000,000 – 100,000,000 tpa	☐ > 100,000,000 tpa
☐ <1 . . . . . >+ tpa (e.g. 10+ ; 100+ ; 10,000+ tpa)		☐ Confidential
Please provide further details if appropriate (e.g. if more than one submission, joint/individual). 1 individual submission (10,000 – 100,000 tpa) and 1 joint submission (1,000 – 10,000 tpa)		

[66] *Please provide here the date when the dissemination site was accessed.*

4.2 Overview of uses

Butylated hydroxytoluene (BHT) is at room temperature (20°C) a white, crystalline, odorless solid (IARC 1986). Food grade should contain not less than 99% BHT. It is insoluble in water but soluble in fats. It is also soluble in ethanol. Physical and chemical properties of BHT are listed in the following table.

Property	**Information**
Molecular weight	180.2a
Melting point	69.8°C at 101.3 kPa
Boiling point	265°C at 101.3 kPa
Flash point	127°C at 1013 hPa
Log *K*ow	5.1 mg/L at 20°C
Water solubility	0.76 mg/L at 20°C Soluble in organic solvents
Vapor pressure	0.011 Pa at 25°C

Non flammable, No explosive, No oxidising (solid)

According to OECD, BHT is used as an antioxidant which finds many applications in a wide variety of industries. It is used in ground vehicle and aviation gasolines; lubricating, turbine, and insulation oils; waxes, synthetic and natural rubbers, paints, plastics, and elastomers. It protects these materials from oxidation during prolonged storage. BHT is used as an antioxidant for food, animal feed, petroleum products, synthetic rubbers, plastics, animal and vegetable oils, and soaps. It serves as an antiskinning agent in paints and inks.

The table including available confidential information on tonnages is given in annex 1.

4.3 Additional information

BHT is a registered antioxidant (EG Antioxidant Directive E321), licensed for food products, animal feed, cosmetics, and packaging material. BHT is also used as an antioxidant for a wide range of applications, including in rubber (mainly during polymerisation), in plastics, in oils, lubricants and fuels.

Exposure of workers to BHT may occur during production, processing and use of the chemical, with the dermal and inhalation routes being the principal routes of exposure.

Many combined RCRs (inhalation + skin contact) have been identified between 0.5 and 1 for workers with the DNELs used by the lead registrant. These DNELS will be compared to Anses DNELs which will be proposed during the evaluation process of the substance.

Concerning the Environment, it must be highlighted that the aquatic PNEC presented in the disseminated information was 3 times higher (4 µg/L) than the one proposed in the OECD SIDS (1.4 µg/L) and no assessment factor to calculate the PNEC sediment and soil was used considering the high Kow value (higher than 5). The proposed assessment should be reconducted considering these points.

Monitoring data are reported in the OECD SIDS. In 1991, a special monitoring program of BHT with a very low determination limit was conducted in German rivers showed the following concentrations (determination limit 0.02 µg/L):
Rhine: < 0.02-0.09 µg/L; 90th percentile 0.08 µg/L
Danube: < 0.02-0.16 µg/L; 90th percentile 0.09 µg/L
Neckar: < 0.02-0.09 µg/L; 90th percentile 0.08 µg/L

A 2003 Swedish study[67] investigated the occurrence of tertiary butylphenols, methylpenols, and long-chain alkylphenols in the Swedish environment. The report indicates that BHT has been previously detected in Swedish influents and effluents from STP (incoming wastewaters 14-42 µg/L; STP effluents 1.2-24 µg/L). Between 70-100% was estimated to derive from households. This paper also indicates that BHT was reported to occur in Dutch STP effluents (concentrations unknown) and in US rivers (max. 0.1 µg/L). In the study, most tertiary butylphenols and the related compounds were detected in sediments, water and sludge, but never in fish. Detection frequencies vary widely in sludge and sediments. BHT was also detected in atmospheric air. Levels in municipal sludge commonly vary by more than two orders of magnitude, and occasionally exceed 1 mg/kg dwt. Nevertheless the updated concentrations in influents and effluents (between 0.1 and 1 µg/L for BHT) are far lower than previously recorded. The report concluded that most substances are efficiently retained or degraded in STP. The levels in sediments downstream municipal STP also span over two orders of magnitude (0.8 – 47 µg/kg dwt).

Further work was recommended by the OECD Cooperative Chemicals Assessment Programme for the following reasons. Releases into the environment during use of BHT and from products containing the substance have to be assumed but are not quantifiable. BHT is not readily biodegradable. Nevertheless, in the environment, BHT is rapidly decomposed forming several, partly unidentified, metabolites. The NOEC from the long-term toxicity to daphnids was 0.07 mg/L, resulting in a PNEC of 0.0014 mg/L. Therefore, the performance of an environmental risk assessment is recommended. Especially the questions concerning exposure, as well as toxicity of the metabolites should be clarified.

Moreover BHT is included in the watch list of the Water Framework Directive (Commission implementing decision (EU) 2015/495 of 20 March 2015) containing 10 substances for which Union-wide monitoring data are to be gathered for the purpose of supporting future prioritisation exercises. In fact for these substances the information available indicated that they may pose a significant risk, at Union level, to or via the aquatic compartment, but monitoring data are insufficient to come to a conclusion on the actual risked posed. The analytical methods indicated for BHT are solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC/MS) and the maximum acceptable method detection limit of 3.16 µg/L.

5 JUSTIFICATION FOR THE RISK MANAGEMENT OPTION

5.1 Need for (further) risk management

Table: SVHC Roadmap 2020 criteria

	Yes	No

[67] Screening tertiary butylphanols, methylphenols, and long-chain alkylphenols in the Swedish environment, IVL Swedish Environmental Reasearch Institute Ltd., IVL report B1594, 2003

a) Art 57 criteria fulfilled?		X (considering available data)
b) Registrations in accordance with Article 10?	x	
c) Registrations include uses within scope of authorisation?	x	
d) Known uses not already regulated by specific EU legislation that provides a pressure for substitution?	x	

5.2 Conclusions on the most appropriate risk management option

Regarding the analysis of the full dataset on BHT, we cannot conclude on ED properties of BHT. First, BHT revealed effects on adrenals and thyroid, which biological significance need to be further evaluated.
Its steric bulk should prevent direct estrogenic and androgenic receptor activation although *in vitro* data show effect of BHT on estrogenic and androgenic receptor, raising questions on its effect on these pathways. There is no animal data to evaluate this MoA. So no conclusion can be drawn regarding the ED mode of action.
Secondly, BHT demonstrated effects on pup survival and pup weight in rat, behavioural effects in reprotoxicity studies.
BHT induced hypersensitivity by oral (diet) and by dermal contact.
There are limited data on immunotoxicity.
The observed hepatocellular tumors, the effects on lung and thyroid in rodent are to be further evaluated. **As a consequence, Anses proposes BHT as a candidate for substance evaluation process.**

Experts from the ED groups agree with France's conclusions based on the current available data (following ED expert group discussions taking place in Helsinki at ECHA the 21-22th of October 2015). Danish EPA supports the testing proposal of an extended one generation (EOGRTS test OECD 443) including B1 cohort, DIT and DNT cohort due to the high sensitivity of sensitive populations such as mothers and children. According to the OECD framework, the EOGRTS is the most appropriate test to determine reprotoxic effects, and conclude on Endocrine Disruptor effects including specific cohorts. The rat model is not the best appropriate model for human (because of the variability in sensitivity of the species to develop tumors of the thyroid). The amphibian test would probably be adapted (LAGDA) to answer environmental challenges and to confirm a possible effect on thyroid in mammals. LAGDA assay (highlighting adverse effects) is prefered to a test on fish as BHT has an activity on thyroid and it has low androgenic or estrogenic activity. HSE UK agency, Danish EPA and AGES Austrian agency confirm that the long-term fish test according to OECD 234 is not relevant. Danish EPA and AGES confirm that LAGDA assay is the most relevant test.

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