

Australian Government

Department of Health Australian Industrial Chemicals Introduction Scheme

Butylated hydroxyanisole and related antioxidants

Evaluation statement

14 January 2022



Table of Contents

Evaluation Statement4
Subject of the Evaluation4
Chemical(s) in this Evaluation4
Reason for the Evaluation4
Parameters of Evaluation4
Summary of Evaluation4
Summary of Introduction, Use and End Use4
Environment5
Conclusions
Supporting Information7
Rationale7
Chemical Identity7
Relevant Physical and Chemical Properties11
Introduction and Use11
Australia11
International12
Existing Australian Regulatory controls12
Environment12
International Regulatory Status12
Canada12
European Union13
Asia13
Environmental Exposure13
Environmental Fate14
Predicted Environmental Concentration (PEC)15

Environmental Effects1	16
Effects on Aquatic Life1	16
Effects on Terrestrial Life1	17
Endocrine Effects/Activity1	18
Predicted No-Effect Concentration (PNEC)1	18
Categorisation of Environmental Hazard1	18
Persistence1	18
Bioaccumulation1	18
Toxicity1	19
GHS Classification of Environmental Hazard1	19
Environmental Risk Characterisation1	19
References2	21

Evaluation Statement

Subject of the Evaluation

Butylated hydroxyanisole and related antioxidants

Chemicals in this Evaluation

Name	CAS Number
Phenol, (1,1-dimethylethyl)-4-methoxy-	25013-16-5
Phenol, 2-(1,1-dimethylethyl)-4-methoxy-	121-00-6
Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	88-32-4
1,4-Benzenediol, 2-(1,1-dimethylethyl)-	1948-33-0
2,5-Cyclohexadiene-1,4-dione, 2-(1,1- dimethylethyl)-	3602-55-9

Reason for the Evaluation

The Evaluation Selection Analysis indicated a potential risk to environment.

Parameters of Evaluation

The chemicals BHA (CAS No. 25013-16-5) and its constituent isomers (CAS Nos. 121-00-6 and 88-32-4), TBHQ (CAS No. 1948-33-0) and TBBQ (CAS No. 3602-55-9) are listed on the Australian Inventory of Industrial Chemicals (the Inventory). The chemicals have been assessed for risks to the environment according to the following parameters:

- Industrial uses listed in the 'Summary of Use' section
- Released to sewage treatment plants as a result of their industrial uses.

The chemicals have been evaluated as a group as they share a common degradation pathway and are present in similar industrial products. BHA degrades through microbial processes to TBHQ and subsequently to TBBQ.

Summary of Evaluation

Summary of Introduction, Use and End Use

There is currently no specific information about the introduction, use and volume end use of the chemical in Australia. Based on international data the chemicals are used as antioxidants in the following industrial products:

- Adhesive and sealant products
- Fuel, oil, fuel oil additives and related products

- Lubricants and Greases
- Personal care products
- Plastic and polymer products
- Paint and coating products.

There is no information available on the domestic use volumes of these substances. Data from international jurisdictions indicate that TBHQ is used in the EU at 100–1000 tonnes annually and in the US at 9065 tonnes (20 000 000 lbs) annually. BHA is used in the EU at 10–100 tonnes annually and in the US at 453 tonnes (1 000 000 lbs) annually.

Environment

Summary of Environmental Hazards

According to domestic environmental hazard thresholds and based on the available data the chemicals are categorised as:

- Persistent (P)
- Not Bioaccumulative (not B)
- Toxic (T)

Environmental Hazard Classification

The chemicals BHA, 2-BHA and 3-BHA satisfy the criteria for classification according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UNECE, 2017) for environmental hazards as follows. This does not consider classification of physical hazards and health hazards:

Environmental Hazard	Hazard Category	Hazard Statement
Hazardous to the aquatic environment (acute / short- term)	Aquatic Acute 2	H401: Toxic to aquatic life
Hazardous to the aquatic environment (long-term)	Aquatic Chronic 2	H411: Toxic to aquatic life with long lasting effects

TBHQ and TBBQ satisfy the criteria for classification according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UNECE, 2017) for environmental hazards as follows. This does not consider classification of physical hazards and health hazards:

Environmental Hazard	Hazard Category	Hazard Statement
Hazardous to the aquatic environment (acute / short- term)	Aquatic Acute 1	H400: Very toxic to aquatic life
Hazardous to the aquatic environment (long-term)	Aquatic Chronic 1	H410: Very toxic to aquatic life with long lasting effects

Summary of Environmental Risk

BHA and TBHQ are synthetic phenolic antioxidants that are expected to have industrial uses within Australia. These chemicals are expected to be released to the aquatic compartment in treated sewage treatment plant (STP) effluent from their industrial uses.

BHA degrades to TBHQ in the environment through microbial demethylation processes. The toxicity endpoints for TBHQ have therefore been considered when characterising the toxicity of BHA in the aquatic environment.

The chemicals in this group are categorised as Persistent, not Bioaccumulative and Toxic. The estimated environmental concentrations of the chemicals do not exceed the PNEC (RQ < 1).

There is inconclusive evidence that BHA and TBHQ are endocrine active chemicals. BHA may cause sexual differentiation in amphibian larvae through oestrogenic activity although this effect was only observed in a single test sample in a single study. The required concentration to elicit this effect is greater than the current environmental concentration of BHA in Australian surface waters.

Conclusions

The conclusions of this evaluation are based on the information described in this statement. Obligations to report additional information about hazards under section 100 of the *Industrial Chemicals Act 2019* apply.

The Executive Director is satisfied that the identified environment risks can be managed within existing risk management frameworks. This is provided that all requirements are met under environmental, workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Supporting Information

Rationale

This evaluation considers the environmental risks associated with the industrial uses of BHA and its two constituent isomers, TBHQ, and a common degradation intermediate of both, TBBQ. BHA and TBHQ belong to a group of substances called synthetic phenol antioxidants (SPA) which are used as preservatives in a range of consumer products to protect against oxidation. BHA and TBHQ are used in large quantities worldwide and are released to the environment from both industrial and non-industrial uses.

The chemicals in this group are linked by a common degradation pathway and common environmental degradant. BHA and its constituent isomers may be transformed into TBHQ by microbial demethylation in the environment. TBHQ can be subsequently oxidised into TBBQ by dissolved oxygen present in environmental waters. In organisms, TBBQ may be reduced back to TBHQ. Therefore, the environmental effects of the common degradant, TBBQ, are likely to be shared by all chemicals in this evaluation.

The evaluation selection analysis (ESA) of the chemicals in this group found that they are potentially of concern to the environment based on the potential for adverse effects to aquatic organisms due to emissions to surface waters in the treated effluent discharged from sewage treatment plants (STP), possible persistent and toxic characteristics, and possible endocrine effects.

Chemical Identity

The chemicals in this group contain a common phenol or quinone structure substituted with a tertiary butyl group. BHA has a methoxy group para- to the phenol hydroxy group. TBHQ has a hydroxy group para to the phenol hydroxy group. The remaining chemicals in this group are structural isomers of BHA or TBBQ which is a *tert*-butyl substituted quinone and is the oxidation product of TBHQ.

Technical BHA (CAS No. 25013-16-5) is typically a 1:9 mixture of its two isomers, 2-BHA and 3-BHA. The isomers differ in the location of the tertiary butyl group relative to the methoxy functional group. The tert-butyl group in 2-BHA is *ortho*- to the methoxy group, while the tert-butyl group in 3-BHA is *meta*- to the methoxy group. The mixture is a result of the non-regioselective methods of manufacture of BHA. BHA is manufactured by either alkylation of *p*-methoxyphenol with isobutylene (CAS No. 150-76-5) or by methylation of TBHQ (Lam, et al., 1979). Steric influence between the tert-butyl and methyl groups involved in either process preferentially yield 3-BHA. As a result, cosmetic grade BHA consists of approximately 90% 3-BHA and 10% 2-BHA (Cosmetic Ingredient Review, 1984).

TBHQ is manufactured by alkylation of hydroquinone (CAS No. 123-31-9) with isobutylene (Cosmetic Ingredient Review, 1986). TBBQ is incidentally produced in products preserved with TBHQ by the reaction of oxygen (Bolton and Dunlap, 2017). The oxidation reaction is likely to be the origin of TBHQs antioxidant function (EFSA, 2004).

Due to the conversion of TBHQ to TBBQ in products and the environment, the exposure of each chemical is not considered separately in this assessment. The release of TBBQ in the environment is directly related to the use of TBHQ. These chemicals are expected to have similar hazard properties in the environment:

CAS No.	25013-16-5
Chemical Name	Phenol, (1,1-dimethylethyl)-4-methoxy-
Synonyms	ВНА
	2(3)-tert-Butyl-4-hydroxyanisole
	Butylated hydroxyanisole
	E 320
Structural Formula	HO CH_3 CH_3 HO CH_3 HO CH_3 H_3C CH_3 H_3C CH_3 H_3C CH_3 CH_3 CH_3 H_3C CH_3
Molecular Formula	C ₁₁ H ₁₆ O ₂
Molecular Weight (g/mol)	181.2533
SMILES	COc1ccc(O)c(c1)C(C)(C)C.O(c(ccc(O)c1C(C)(C)C)c1) C



CAS No.	88-32-4
Chemical Name	Phenol, 3-(1,1-dimethylethyl)-4-methoxy-
Synonyms	2-BHA 2-tert-Butyl-4-hydroxyanisole
Structural Formula	$H_{3}C$ $H_{3}C$ $H_{3}C$ $H_{3}C$ CH_{3} $H_{3}C$
Molecular Formula	C ₁₁ H ₁₆ O ₂
Molecular Weight (g/mol)	181.2533
SMILES	O(c(c(cc(O)c1)C(C)(C)c1)C

CAS No.	1948-33-0
Chemical Name	1,4-Benzenediol, 2-(1,1-dimethylethyl)-
Synonyms	TBHQ tert-butylhydroquinone t-Butyl hydroquinone E319
Structural Formula	HO H ₃ C H ₃ C CH ₃
Molecular Formula	C ₁₀ H ₁₄ O ₂
Molecular Weight (g/mol)	166.22
SMILES	CC(C)(C)C1=C(C=CC(=C1)O)O

CAS No.	3602-55-9
Chemical Name	2,5-Cyclohexadiene-1,4-dione, 2-(1,1-dimethylethyl)-
Synonyms	TBBQ tert-butylbenzoquinone
Structural Formula	CH ₃ CH ₃
Molecular Formula	$C_{10}H_{12}O_2$
Molecular Weight (g/mol)	164.20
SMILES	O=C1C=CC(=O)C(=C1)C(C)(C)C
Chemical Description	-

Relevant Physical and Chemical Properties

The measured physical and chemical property data for BHA, TBHQ and TBBQ were retrieved from the databases included in the OECD QSAR Toolbox (LMC, 2020), dossiers submitted under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (ECHA, 2015a) and from peer-reviewed scientific literature (Fischer and Henderson, 1985). The calculated values for BHA, TBHQ and TBBQ were obtained using standard quantitative structure (property) relationships (US EPA, 2020):

Chemical	BHA	TBHQ	TBBQ
Physical Form	solid	solid	solid
Melting Point	57–59 °C (exp.)	107°C (exp.)	58–59 °C (exp .)
Boiling Point	240 °C (exp.)	295°C (exp.)	269.8 °C (calc.)
Vapour Pressure	0.311 Pa (exp.)	0.007 (calc.)	1.0 Pa (calc.)
Water Solubility	213 mg/L (exp.)	2 887 mg/L (exp.)	1 045 mg/L (calc.)
Henry's Law Constant	0.119 Pa-m³/mole (calc.)	0.0004 Pa-m ³ /mole (calc.)	0.16 Pa-m³/mole (calc.)
lonisable in the Environment?	no	no	no
рКа	10.6 (calc.)	10.7 (exp.)	-
log K _{ow}	2.82 (exp)	1.5 (exp.)	2.16 (calc.)

The acid dissociation constants (pK_a) for TBHQ and BHA indicates they will not be ionised in the environmental pH range of 4–9.

The logarithmic octanol-water partition coefficients (log K_{OW}) of the chemicals in this group indicate they have a low lipophilicity and will likely remain in the water compartment following release to surface waters.

Introduction and Use

Australia

No specific Australian industrial use, import or manufacturing information has been identified for the chemicals in this group.

BHA and TBHQ are listed under Schedule 15 of the Australia New Zealand Food Standards Code for use as a food additive (Commonwealth of Australia, 2021). The use of these chemicals as food additives is outside of the scope of this assessment.

BHA is used as an excipient in therapeutic goods (TGA, 2021). The use of BHA in pharmaceuticals is outside the scope of this assessment.

International

BHA and TBHQ are used as antioxidants in a wide range of products. Antioxidants are chemicals added to products to protect from the effects of oxidation while in storage. They are used in low concentrations ($\leq 0.1-1\%$) in consumer goods such as cosmetics and fragrances (Pubchem, 2021).

BHA and TBHQ are also used for the preservation of materials such as rubber products, polymers and coatings. They are used in food packaging as they are accepted as safe for human and animal consumption at low levels (Cosmetic Ingredient Review, 1984; 1986).

Both chemicals are used in biodiesel, lubricants and greases as a stabilizer to prevent gum formation (US EPA, 2016; Varatharajan and Pushparani, 2017).

In the European Union (EU), BHA is registered for use at a volume between 10 and 100 tonnes per annum, TBHQ at a volume of 100 to 1000 tonnes per annum (ECHA, 2020).

In the United States of America (USA) BHA and TBHQ are listed on the U.S. HPV Challenge Program Chemical List as being manufactured or imported into the United States in amounts ≤453 tonnes per year (BHA) and 453–9065 tonnes per year (TBHQ) in 2016 (US EPA, 2021a). BHA production was less than 226 tonnes in 2006 (National Toxicology Program, 2016).

TBHQ was manufactured or imported in quantities of 1–1000 tonnes per annum in 2018 (NITE, 2020).

TBHQ is identified as a high-production volume (HPV) chemical by the OECD which indicates that more than 1000 tonnes is produced per year by at least one member country (OECD, 2009).

Available information indicates TBBQ is used only as a research chemical (Merck, 2021).

Existing Australian Regulatory controls

Environment

The use of the chemicals in this group is not subject to any specific national environmental regulations.

International Regulatory Status

Canada

TBHQ, BHA, 2-BHA and 3-BHA are listed on the Canadian Domestic Substances List (DSL). The Screening Assessment for BHA concluded that it is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long term harmful effect on the environment (Environment Canada, 2010).

European Union

BHA is currently undergoing evaluation under the Community Rolling Action Plan (CoRAP) (ECHA, 2016). The chemical qualifies for CoRAP evaluation based on its wide dispersive release, exposure to consumers and sensitive populations and potential reprotoxic and endocrine activity (ECHA, 2015b).

Asia

BHA is currently listed in Japan as a Type II Monitoring Chemical Substances – chemicals suspected of posing a risk to human health and Type III Monitoring Chemical Substances – chemicals that pose a risk to flora and fauna.

Environmental Exposure

BHA and TBHQ are used internationally as antioxidants in a wide range of consumer products, such as cosmetics, fragrances, coatings, food packaging, lubricants, and greases. The use of the chemicals in these products in Australia is not expected to differ greatly from international use.

Chemicals used in cosmetics and fragrances will typically be released to wastewater following their normal use. Treatment of this wastewater in STPs will remove a fraction of the quantity of these chemicals in influents, depending on the efficiency of degradation and partitioning processes. Based on the water solubility, volatility, and low lipophilicity of the chemicals in this group, they are unlikely to be removed in STPs by partitioning processes (volatilisation or adsorption to sludge). However, up to 41% may be removed by biodegradation (Struijs, 1996). Release to the environment in effluent following treatment at STPs is expected to be the major emission pathway for these chemicals.

BHA and TBHQ are also used as antioxidants in food packaging, rubber and coating products (Pubchem, 2021). Release of these chemicals into the environment may occur from their migration to the surface of these articles and subsequent release due to abrasion and wear from normal use.

Available international information indicates that BHA and TBHQ are used in petroleum products such as biodiesel, lubricants, and greases. Emissions to the environment due to accidental spills and inappropriate disposal may occur according to the standard exposure scenario for these use patterns (OECD, 2004).

As a research chemical, TBBQ is not expected to have emissive industrial use and therefore any TBBQ in the environment is expected to be a result of the degradation of TBHQ.

The chemicals in this group are used in large volumes as preservatives in food. Available evidence indicates these chemicals are not completely absorbed by humans from dietary exposure and are likely released in substantial quantities to STPs from this source (Astill, et al., 1962; FAO/WHO, 1975). A significant proportion of the chemical released to the environment may therefore originate from these non-industrial sources.

Environmental Fate

Partitioning

The chemicals in this group are expected to primarily partition to water after release to the environment from their industrial use.

The chemicals in this group are not expected to appreciably volatilise to air from surface waters. BHA and TBBQ are moderately volatile from water and TBHQ is slightly volatile from water based on calculated Henry's Law constants. Volatility modelling indicates that the chemicals have long volatilisation half-lives from model rivers and lakes (half-lives > 28 days) (US EPA, 2008).

The measured logarithmic organic carbon normalised soil adsorption coefficient (log K_{oc}) of 1.45 for TBHQ indicates a very high mobility in soil (REACH, 2021a). The moderate log K_{oc} values for BHA (log K_{oc} = 3.15, measured) and TBBQ (log K_{oc} = 2.46, calculated) indicate these chemicals have a low mobility in soil (REACH, 2021b; US EPA, 2020).

Degradation

There is insufficient evidence to determine the persistence hazard of the chemicals in this group. As a precaution, the chemicals are categorised as persistent in water and sediments until sufficient evidence is made available. The chemicals will undergo degradation to some extent in the environment as demonstrated by STP removal studies.

The chemicals in this group are linked by a common degradation pathway. BHA and its constituent isomers may be transformed into TBHQ by microbial demethylation in the environment. TBHQ may subsequently be oxidised into TBBQ. In organisms, TBBQ may be reduced back to TBHQ.

Ready biodegradability tests conducted according to OECD TG 301D for BHA and TBHQ found 34% and 47% degradation respectively over 28 days as measured by biological oxygen demand (ECHA, 2015a). There is uncertainty around the validity of these results, as interconversion between TBHQ and TBBQ can consume oxygen without resulting in mineralisation of the test substance. The results of these studies were not corrected for abiotic degradation using an abiotic control as detailed in the OECD TG301 guidance document (OECD, 1992). The results of these tests may therefore overestimate the biodegradability of BHA and TBHQ.

BHA is expected to degrade to TBHQ in the environment. BHA has been shown to degrade to TBHQ in in vitro assays simulating metabolism. This process occurs by O-demethylation mediated by cytochrome P450, an enzyme ubiquitous in organisms in the environment (Bampidis, et al., 2019). OASIS Catalogic biodegradation modelling indicates that the majority of BHA will be converted to TBHQ after 28 days (LMC, 2011).

TBHQ abiotically degrades to TBBQ in oxygenated water. In vitro measurements on the oxidation process in a buffered neutral pH solution indicate a half-life of 6 h (Ooi, et al., 2013).

The chemicals in this group are rapidly degraded in the atmosphere, however this is not expected to be a major degradation pathway. Calculated half-lives for photochemical oxidation of the chemicals in this group are 7.1, 5.0 and 9.5 hours for BHA, TBHQ and TBBQ

respectively (LMC, 2020). The chemicals are slightly to moderately volatile and do not significantly partition to the atmosphere.

Photodegradation in surface waters may be a possible dissipation pathway for the chemicals in this group. A study of BHA in soybean oil shows that the chemical undergoes photodegradation when exposed to sunlight (Mihara, et al., 1974). A similar study using TBHQ found that fluorescent light accelerated the auto-oxidation of the chemical (Cosmetic Ingredient Review, 1986). Although the environmental significance of these findings are uncertain, they indicate a possible light mediated degradation mechanism for BHA and TBHQ.

No studies on the degradation of BHA or TBHQ in soil or sediment have been identified.

Bioaccumulation

The chemicals are expected to have low bioaccumulation potential in aquatic organisms.

Measured and calculated bioconcentration factors (BCF) for the chemicals in this group are below the criteria for categorisation for bioaccumulation (BCF \leq 2000 L/kg) (EPHC, 2009). Tests of the bioconcentration potential of BHA in rice fish (*Oryzias latipes*) were observed to be in the range of 16–21 L/kg (NITE, 2020). For TBHQ the bioaccumulation range in rice fish was 8.1–18 L/kg (NITE, 2020). Calculated BCF values of 40.21 L/kg for BHA, 2.72 L/kg for TBHQ and 12.3 L/kg for TBBQ after accounting for biotransformation effects were obtained through predictive models (US EPA, 2020).

Environmental Transport

The chemicals in this group are not expected to undergo long range transport.

The chemicals in this group have short atmospheric half-lives and do not significantly partition to the atmosphere. They are also expected to slowly degrade in environmental surface waters, which is expected to limit their potential to undergo long range transport.

Predicted Environmental Concentration (PEC)

The PECs for BHA and TBHQ in inland surface waters are 1 microgram per litre (μ g/L) and 0.079 μ g/L respectively.

Australian environmental monitoring data are not available for the chemicals in this group.

BHA and TBHQ are expected to enter the environment from STP effluent. Initial estimations using a standard STP exposure model indicate 39-41% removal of the chemicals from wastewater by biodegradation (EPHC, 2009; Struijs, 1996). Assuming 100 tonnes per annum introduction of BHA and TBHQ and 100% release of these chemicals to sewage, a predicted environmental concentration of 32.02–34.2 μ g/L is estimated.

International monitoring data suggest that these concentrations may be overestimates. A monitoring study of alkylphenol concentrations in environmental media and STPs in Sweden found BHA and TBHQ in surface waters, stormwaters and STP influent and effluent samples (Remberger, et al., 2004). BHA was found at a maximum concentration of 0.29 μ g/L in STP influent, and a maximum concentration of 0.035 μ g/L in effluent. TBHQ was found at maximum concentrations of 0.079 μ g/L in STP influent, and 0.014 μ g/L in STP effluent. The level of treatment in these STPs was not specified, so it is unknown if these removal rates

are realistic for Australia. The influent concentration for TBHQ is conservatively used as representative of surface water concentrations in Australia.

Another monitoring study from 3 STPs in the USA found BHA in effluent after secondary treatment at up to 1 μ g/L (Soliman, et al., 2007). This value is used as representative of BHA surface water concentrations in Australia

Based on these monitoring data, maximum concentrations of BHA and TBHQ in treated STP effluent in Australia are estimated to be 1 μ g/L and 0.079 μ g/L respectively.

TBBQ is only expected to be present in the environment due to the degradation of THBQ, therefore the maximum concentration of TBBQ is taken as $0.079 \mu g/L$.

Environmental Effects

The chemicals in this group exhibit toxic effects on aquatic organisms in both short- and long-term exposure scenarios. BHA, TBHQ and TBBQ have a specific mode of toxicity related to their chemical reactivity. Quinones such as TBHQ/TBBQ have the potential to bind to thiol, amine and hydroxyl groups in the proteins of enzymes, which may be inactivated due to this reaction (El-Najjar N, et al., 2011). BHA has a specific mode of toxicity common to phenols. It potentially acts by uncoupling oxidative phosphorylation, inhibiting the respiration process in both animal and plants cells (Wynn and Fore, 1965).

Effects on Aquatic Life

TBHQ undergoes oxidation to TBBQ in oxygenated water, in processes that cannot be controlled in the test solutions of standard OECD ecotoxicity tests. The organisms in this test are therefore assumed to be exposed to both TBHQ and TBBQ in various proportions during the progression of the test. TBBQ concentration will increase as the test progresses, however, metabolic processes in vivo have been observed to reduce TBBQ back to TBHQ (EI-Najjar Nahed, et al., 2011). Therefore, it is assumed the toxicity endpoints for TBHQ are indicative of the toxic effects exhibited by TBBQ exposure.

Acute toxicity

The following median lethal concentration (LC50) and median effective concentrations (EC50s) for model organisms across three trophic levels were obtained from databases in QSAR toolbox (LMC, 2020):

Taxon	Endpoint	Method
Fish	BHA: 96 h LC50 = 1.56 mg/L TBHQ: 96 h LC50 = 0.3 mg/L	<i>Danio rerio</i> (zebrafish) Static, nominal concentration OECD TG 203
Invertebrate	BHA: 48 h EC50 = 2.3 mg/L TBHQ: 48 h EC50 = 0.57 mg/L	<i>Daphnia magna</i> (water flea) Static, nominal concentration OECD TG 202
Algae	BHA: 72 h EC50 = 5.2 mg/L	Pseudokirchneriella subcapitata (green algae) Growth rate Static, nominal concentration OECD TG 201
	TBHQ: 72 h EC50 = 9.3 mg/L	<i>Desmodesmus subspicatus</i> (green algae) Static, nominal concentration OECD TG 201

Chronic toxicity

The following no observed effect concentration (NOEC) for algae was obtained from the Japan Chemicals Collaborative Knowledge database (J-CHECK, 2021):

Taxon	Endpoint	Method
Algae	BHA: 72 h NOEC = 0.25 mg/L	<i>Pseudokirchneriella subcapitata</i> (green algae) Growth rate OECD TG 201

Effects on Terrestrial Life

BHA and TBHQ/TBBQ have inhibitory effects on microbial organisms.

TBBQ has a measured minimum inhibitory concentration (MIC) of 8 mg/L against the gram positive bacteria *Staphylococcus aureus* (Ooi, et al., 2013). Similar MIC tests performed with TBHQ showed a 2-hour lag in activity.

The minimum BHA concentration required to inhibit several microorganisms has been demonstrated to be 125 mg/L (Cosmetic Ingredient Review, 1984).

Endocrine Effects/Activity

BHA and TBHQ demonstrate endocrine activity, however the ecological significance of these effects at expected Australian environmental concentrations remains uncertain.

BHA may affect sexual differentiation in amphibians. A study on tadpoles of *Xenopus laevis* (African clawed frog) showed evidence of feminisation during larval development (Kloas, et al., 1999). Larvae exposed to 18.1 μ g/L during a period of 12 weeks resulted in a slightly higher proportion of female phenotypes when compared to control test solutions. This effect was not observed in the 1.8 μ g/L concentration sample.

ToxCast high-throughput screening for endocrine activity identified 3-BHA and TBHQ as having very weak oestrogenic or androgenic activity (Browne, et al., 2015; Kleinstreuer, et al., 2017; US EPA, 2021b).

Predicted No-Effect Concentration (PNEC)

The PNECs for BHA and TBHQ/TBBQ are 2.5 μ g/L and 3 μ g/L respectively.

BHA will degrade to TBHQ when released to the environment and will contribute to levels of environmental TBHQ. As a precaution, the estimated PNEC for BHA is obtained by considering the toxicity data available for its toxic degradant, TBHQ. However, incomplete chronic data are available for TBHQ, and the lowest available endpoint for all chemicals is a chronic value for BHA. PNECs derived from the lowest acute and chronic endpoints for both chemicals were compared and the lowest taken.

A PNEC of 2.5 μ g/L was derived from the chronic toxicity value of BHA for algae (72 h EC50 = 0.25 mg/L). An assessment factor of 100 was selected as chronic data is available for one trophic level.

A PNEC of 3 μ g/L was derived from the acute toxicity value of TBHQ for fish (96 h LC50 = 0.3 mg/L). An assessment factor of 100 was selected as no chronic data is available.

Categorisation of Environmental Hazard

The categorisation of the environmental hazards of the assessed chemical according to domestic environmental hazard thresholds is presented below:

Persistence

Persistent (P). Based on the uncertainty of the available ready biodegradability tests and in the absence of other information, the chemicals are categorised as Persistent.

Bioaccumulation

Not Bioaccumulative (Not B). Based on low measured bioconcentration factors (BCF) in fish, BHA and TBHQ are categorised as Not Bioaccumulative.

Toxicity

Toxic (T). Based on available ecotoxicity values below domestic thresholds, TBHQ and TBBQ are categorised as Toxic. BHA is categorised as toxic based on endpoints of its toxic degradant, TBHQ.

GHS Classification of Environmental Hazard

Based on the available data, BHA, 2-BHA, 3-BHA are categorised as Acute category 2 (H401) and Chronic Category 2 (H411) under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS).

Incomplete chronic aquatic toxicity data are available. Therefore, according to the GHS guidance on classification of aquatic hazards, the chronic aquatic hazard of this chemical may be based on either the acute or chronic ecotoxicity endpoints choosing the most stringent outcome. In this case, the outcome was the same, so the long-term aquatic hazard of these chemicals was classified based on the measured chronic algal ecotoxicity endpoint.

Based on the available data, TBHQ and TBBQ are categorised as Acute category 1 (H400) and Chronic Category 1 (H410) under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS).

Incomplete chronic aquatic toxicity data are available. Therefore, according to the GHS guidance on classification of aquatic hazards, the chronic aquatic hazard of this chemical may be based on the acute ecotoxicity endpoints. In this case, the long-term aquatic hazard of these chemicals was classified based on the measured acute fish ecotoxicity endpoint.

Environmental Risk Characterisation

Based on the PEC and PNEC values determined above, the following Risk Quotient (RQ = PEC ÷ PNEC) has been calculated for release of BHA and TBHQ into rivers:

Chemical	PEC (µg/L)	PNEC (µg/L)	RQ
BHA	1	2.5	0.4
TBHQ/TBBQ	0.079	3	0.02

An RQ of less than 1 indicates that BHA and TBHQ are not expected to pose a risk to the environment based on the estimated emissions, as environmental concentrations are below the levels likely to cause harmful effects.

There is some evidence that BHA and TBHQ have endocrine activity, however the ecological significance remains uncertain based on low environmental concentrations and the weak endocrine response in screening assays. The lowest observed effect concentration for endocrine effects is two orders of magnitude above the predicted environmental concentration. Further evaluation may be required if more information becomes available characterising the extent of the endocrine risk, or monitoring data indicate higher chemical concentrations in Australian surface waters than the PEC estimated in this evaluation.

Industrial use of BHA and TBHQ are not expected to pose an unreasonable risk to soil organisms as the chemicals partition predominantly to the aquatic compartment.

Uncertainty

This evaluation was conducted based on a set of information that may be incomplete or limited in scope. Some relatively common data limitations can be addressed through use of conservative assumptions (OECD, 2019) or quantitative adjustments such as assessment factors (OECD, 1995). Others must be addressed qualitatively, or on a case-by-case basis (OECD, 2019).

The most consequential areas of uncertainty for this evaluation are:

- The proposed Australian environment concentrations in this assessment are reliant on consistency between Australian and International per-capita use and release patterns. Consequently, this assessment does not account for internal regulatory, economic, social or other pressures that may influence emission scenarios now or in the future. The concentration values used of 1 µg/L for BHA and 0.079 µg/L TBHQ/TBBQ have been selected as the best conservative estimate based on available information at this time.
- Significant environmental releases of these chemicals may occur from non-industrial sources. The chemicals have a high-volume use in foodstuffs. There is insufficient information available to determine what percentage of the release is from industrial sources, therefore, it is likely that the PEC used in this evaluation is overestimated for industrial releases.
- The persistence of the chemicals in this group is likely to be overestimated. Available biodegradability studies do not account for abiotic oxygen demand in the study design as detailed in the OECD TG301 guidance document. Photodegradation mechanisms have also been identified but not quantified, indicating another possible source of overestimation of persistence in the Australian riverine environment. The chemicals have been screened as having endocrine activity, but the extent of these effects have not been fully characterised, and available data appear contradictory. In vitro assays indicate very weak endocrine activity; however, a non-ideal in vivo study indicates sexual differentiation effects in a single test sample for one chemical. The predicted Australian environmental concentration is below the level of effect identified in literature, although it highlights the need for further studies as in vitro studies may not be able to account for organism level effects.

References

Astill BD, Mills J, Fassett DW, Roudabush RL and Terhaar CJ (1962). Food Additives Metabolism, Fate of Butylated Hydroxyanisole in Man and Dog. *Journal of Agricultural and Food Chemistry*, **10**(4), pp 315-319.

Bampidis V, Azimonti G, Bastos M, Christensen H, Dusemund B, Kouba M, Durjava M, López-Alonso M, López S, Marcon F, Mayo B, Pechova A, Petkova M, Ramos F, Sanz Y, Villa R, Woutersen R, Aquilina G, Bories G and Innocenti M (2019). Safety of butylated hydroxy anisole (BHA) for all animal species. *EFSA journal. European Food Safety Authority*, **17**, pp e05913.

Bolton JL and Dunlap T (2017). Formation and Biological Targets of Quinones: Cytotoxic versus Cytoprotective Effects. *Chemical Research in Toxicology*, **30**(1), pp 13-37.

Browne P, Judson RS, Casey WM, Kleinstreuer NC and Thomas RS (2015). Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model. *Environ Sci Technol*, **49**(14), pp 8804-14.

Commonwealth of Australia (2021). *Australia New Zealand Food Standards Code* -*Schedule 15 - Substances that may be used as food additives - F2017C00331.* Commonwealth of Australia, Canberra Australia. Accessed February at <u>https://www.legislation.gov.au</u>.

Cosmetic Ingredient Review (1984). Final Report on the Safety Assessment of Butylated Hydroxyanisole. *Journal of the American College of Toxicology*, **3**(5), pp 83-146.

Cosmetic Ingredient Review (1986). 3 Final Report on the Safety Assessment of t-Butyl Hydroquinone. *International Journal of Toxicology*, **5**, pp 329 - 351.

ECHA (2015a). *Registered Substances Database*. European Chemicals Agency, Helsinki, Finland. Accessed 1 April at <u>http://echa.europa.eu/information-on-chemicals/registered-substances</u>.

ECHA (2015b). Justification for the selection of a substance for CoRAP inclusion. Accessed February 2021 at <u>https://echa.europa.eu/documents/10162/13628/corap_justification_246-563-8_fr_15256_en.pdf/ab031c03-6e40-c32f-4e3f-f5ef6b1c7dad</u>.

ECHA (2016). *Substance evaluation - CoRAP*. European Chemicals Agency, Helsinki, Finland. Accessed 2 February 2016 at <u>http://echa.europa.eu/information-on-</u><u>chemicals/evaluation/community-rolling-action-plan/corap-table</u>.

ECHA (2020). *Registered substances*. Helsinki, Finland. Accessed July at <u>https://echa.europa.eu/information-on-chemicals/registered-substances</u>.

EFSA (2004). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to tertiary-Butylhydroquinone (TBHQ) European Food Safety Authority, Parma, Italy.

El-Najjar N, Gali-Muhtasib H, Ketola R, Vuorela P, Urtti A and Vuorela H (2011). The chemical and biological activities of quinones: overview and implications in analytical detection. *Phytochemistry Reviews*, **10**, pp 353-370.

El-Najjar N, Gali-Muhtasib H, Ketola RA, Vuorela P, Urtti A and Vuorela H (2011). The chemical and biological activities of quinones: overview and implications in analytical detection. *Phytochemistry Reviews*, **10**(3), pp 353.

Environment Canada (2010). *Screening Assessment for the Challenge - Phenol, (1,1-dimethylethyl)-4-methoxy-*. Accessed February at <u>https://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=6E4A53B5-1</u>.

EPHC (2009). *Environmental Risk Assessment Guidance Manual for Industrial Chemicals*. Environment Protection and Heritage Council, Canberra, Australia. Accessed 9 December 2013 at <u>http://www.scew.gov.au/resource/chemical-risk-assessment-guidance-manuals</u>.

FAO/WHO (1975). *Toxicological evaluation of some food colours, thickening agents, and certain other substances*. Geneva, Switzerland. Accessed August 2021 at https://inchem.org/documents/jecfa/jecmono/v08je11.htm.

Fischer A and Henderson GN (1985). Oxidation of Hydroquinones, Catechols, and Phenols using Ceric Ammonium Nitrate and Ammonium Dichromate Coated on Silica: An Efficient and Convenient Preparation of Quinones. *Synthesis*, **1985**(6/7), pp 641-643.

J-CHECK (2021). *Japan Chemicals collaborative Knowledge database*. National Institute of Technology and Evaluation, Tokyo, Japan. Accessed 14 May at https://www.nite.go.jp/chem/jcheck/search.action?request_locale=en.

Kleinstreuer NC, Ceger P, Watt ED, Martin M, Houck K, Browne P, Thomas RS, Casey WM, Dix DJ, Allen D, Sakamuru S, Xia M, Huang R and Judson R (2017). Development and Validation of a Computational Model for Androgen Receptor Activity. *Chemical research in toxicology*, **30**(4), pp 946-964.

Kloas W, Lutza I and Einspanier R (1999). Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals in vitro and in vivo. *Science of The Total Environment*, **225**(1-2), pp 59-68.

Lam LK, Pai RP and Wattenberg LW (1979). Synthesis and chemical carcinogen inhibitory activity of 2-tert-butyl-4-hydroxyanisole. *J Med Chem*, **22**(5), pp 569-71.

LMC (2011). OASIS Catalogic, v 5.10.9. Laboratory of Mathematical Chemistry. University "Prof. Dr. Assen Zlatarov", Burgas, Bulgaria.

LMC (2020). *The OECD QSAR Toolbox for Grouping Chemicals into Categories*, v 3.3. Laboratory of Mathematical Chemistry, University "Prof. Dr. Assen Zlatarov", Burgas, Bulgaria. Available at <u>http://oasis-lmc.org/</u>.

Merck (2021). 2-*tert-Butyl-1,4-benzoquinone*. Accessed 13 May at https://www.sigmaaldrich.com/catalog/product/aldrich/429074?lang=en®ion=AU.

Mihara M, Kondo T and Tanabe H (1974). Studies on Photolysis of Food Additives (II)

Photolysis of Butyl Hydroxy Anisole in Oil and Fat. *Food Hygiene and Safety Science* (*Shokuhin Eiseigaku Zasshi*), **15**(4), pp 276-279_1.

National Toxicology Program (2016). *Report on Carcinogens, Fourteenth Edition*. U.S. Department of Health and Human Services PHS, Research Triangle Park, NC.

https://ntp.niehs.nih.gov/whatwestudy/assessments/cancer/roc/index.html?utm_source=dire_ct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=roc.

NITE (2020). *Japan CHEmicals Collaborative Knowledge Database (J-CHECK)*. National Institute of Technology and Evaluation, Tokyo, Japan. Accessed July at http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en.

OECD (1992). *Test No. 301: Ready Biodegradability*. The Organisation for Economic Cooperation and Development, Paris, France. Accessed August at <u>https://read.oecd-</u> <u>ilibrary.org/environment/test-no-301-ready-biodegradability_9789264070349-en#page1</u>.

OECD (1995). *Guidance document for aquatic effects assessment*. Organisation for Economic Co-operation and Development, Paris.

OECD (2004). *Emission Scenario Document on Lubricants and Lubricant Additives*. Paris France. Accessed March at <u>https://www.oecd-ilibrary.org/environment/lubricants-and-lubricant-additives 9789264221154-en</u>.

OECD (2009). *The 2009 OECD List of High Production Volume Chemicals*. Organisation for Economic Co-operation and Development, Paris, France. Accessed March at <u>https://hpvchemicals.oecd.org</u>.

OECD (2019). Guiding Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment, Series on Testing and Assessment No. 311, Environment, Health and Safety Division, Environment Directorate. <u>http://www.oecd.org/chemicalsafety/risk-assessment/guiding-principles-and-key-elements-for-establishing-a-weight-of-evidence-for-chemical-assessment.pdf</u>.

Ooi N, Chopra I, Eady A, Cove J, Bojar R and O'Neill A (2013). Antibacterial activity and mode of action of tert-butylhydroquinone (TBHQ) and its oxidation product, tert-butylbenzoquinone (TBBQ). *Journal of Antimicrobial Chemotherapy*, pp 1297-1304.

Pubchem (2021). *PubChem Compound Summary* National Library of Medicine. Accessed February at <u>https://pubchem.ncbi.nlm.nih.gov/</u>.

REACH (2021a). *REACH registration dossier for 2-tert-butylhydroquinone CAS RN:1948-33-0*. Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Brussels, Belgium. Accessed August 2021 at <u>https://echa.europa.eu/registration-dossier/-/registered-dossier/5612</u>.

REACH (2021b). *REACH registration dossier for tert-butyl-4-methoxyphenol CAS RN:25013-16-5.* Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Brussels, Belgium. Accessed August 2021 at <u>https://echa.europa.eu/registration-dossier/-/registered-dossier/15988/5/5/2</u>.

Remberger M, Kaj L, Cousins A, Sternbeck J, Kvernes E and Brorström-Lundén E (2004). Screening tertiary butylphenols, methylphenols, and long-chain alkylphenols in the Swedish environment.

Soliman MA, Pedersen JA, Park H, Castaneda-Jimenez A, Stenstrom MK and Suffet IH (2007). Human pharmaceuticals, antioxidants, and plasticizers in wastewater treatment plant and water reclamation plant effluents. *Water Environ Res*, **79**(2), pp 156-67.

Struijs J (1996). *SimpleTreat 3.0: a model to predict the distribution and elimination of chemicals by sewage treatment plants*. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

TGA (2021). *Australian Register of Therapeutic Goods*. Therapeutic Goods Administration, Canberra, Australia. <u>https://www.tga.gov.au/searching-australian-register-therapeutic-goods-artg</u>.

UNECE (2017). *Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Seventh Revised Edition*. Geneva, Switzerland. Accessed February at https://www.unece.org/trans/danger/publi/ghs/ghs rev03/03files e.html.

UNEP (1987). *The Montreal Protocol on Substances that Deplete the Ozone Layer*. United Nations Environment Programme, Ozone Secretariat, Nairobi, Kenya. Accessed July at http://ozone.unep.org/.

UNEP (2001). *The Stockholm Convention on Persistent Organic Pollutants*. Châtelaine, Switzerland. Accessed July at <u>http://www.pops.int/</u>.

UNEP & FAO (1998). Rotterdam Convention on the Prior Informed Consent Procedure for *Certain Hazardous Chemicals and Pesticides in International Trade*. Châtelaine, Switzerland. Accessed at <u>http://www.pic.int/</u>.

US EPA (2008). *Estimations Programs Interface (EPI) Suite™ for Microsoft Windows*®, v 4.10. United States Environmental Protection Agency, Washington DC, USA. Available at <u>http://www.epa.gov/oppt/exposure/pubs/episuite.htm</u>.

US EPA (2016). *Chemview (US CDR data)*. United States Environmental Protection Agency, Washington D.C., USA. Accessed 13 May at <u>https://chemview.epa.gov/chemview</u>.

US EPA (2020). *Estimation Programs Interface (EPI) Suite™ for Microsoft Windows*®, v 4.10. United States Environmental Protection Agency, Washington DC, USA. Available at <u>https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface</u>.

US EPA (2021a). *Chemical Data Reporting*. United States Environmental Protection Agency, Washington DC, USA. Accessed March at <u>https://www.epa.gov/chemical-data-reporting/access-cdr-data</u>.

US EPA (2021b). *CompTox Chemicals Dashboard*. United States Environmental Protection Agency, Durham, USA. Accessed March at <u>https://comptox.epa.gov/dashboard</u>.

Varatharajan K and Pushparani DS (2017). Screening of antioxidant additives for biodiesel fuels. *Renewable and Sustainable Energy Reviews*, **82**.

Wynn J and Fore W (1965). The Effect of Hindered Phenols on Mitochondrial Oxidative Phosphorylation. *Journal of Biological Chemistry*, **240**(4), pp 1766-1771.