Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment

Protocol for the risk assessment of butylated hydroxytoluene (BHT)

From the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food and Environment

From the Norwegian Scientific Committee for Food and Environment (VKM)
Protocol for the risk assessment of butylated hydroxytoluene (BHT)

The Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food and Environment
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Protocol for the risk assessment of butylated hydroxytoluene (BHT)

Preparation of the protocol

A project group prepared this protocol for the risk assessment of BHT. The project group consisted of two VKM members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics, one external expert, and to employees of the VKM secretariat. The VKM Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics evaluated and approved the final protocol drafted by the project group.

Assessed and approved

Members of the project group that contributed to the drafting of the protocol (in alphabetical order after chair of the project group):

Trine Husøy - Chair of the project group and member of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health

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Members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics that contributed to and approved the protocol:

In addition to Trine Husøy and Jens Rohloff, these were (in alphabetical order):

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Acknowledgment

VKM would like to thank librarian Marita Heinz (Norwegian Institute of Public Health), for assistance with the literature search.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

Table of Contents

1 Introduction

Butylated hydroxytoluene (BHT) is a synthetic antioxidant authorised as a food additive in the EU (E321). BHT is also used in animal feed, cosmetics, food contact materials and pharmaceuticals.

Several expert committees have previously assessed BHT, and an ADI of 0.25 mg/kg body weight (bw) per day was established in 2012 by EFSA (EFSA, 2012). The ADI was based on a NOAEL of 25 mg/kg bw per day derived from two 2-generation studies in rats and an uncertainty factor of 100.

To our knowledge, risk assessments including aggregated exposure estimates for BHT from several sources and exposure pathways have not been performed. As there is extensive use of BHT, there is a need for an updated risk assessment for the Norwegian population. Therefore, the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food and Environment (VKM) has self-initiated a risk assessment of BHT that includes exposure estimations comprising several sources and exposure pathways.

The protocol presented in this document describes the methodology that will be applied for the BHT risk assessment.

1.1 Terms of reference

The terms of reference for the risk assessment of BHT, is to assess whether BHT constitutes a health risk to the Norwegian population, and to assess which groups in the population that have the highest exposure.

The target group for the risk assessment is the Norwegian population, both sexes, all age groups (infants, children, adolescents and adults).

2 Problem formulation

2.1 Objectives of the risk assessment

The overall aim of the risk assessment is to assess whether BHT exposure from foods, cosmetics, pharmaceuticals, indoor dust and indoor air constitutes a health risk to the Norwegian population, to identify the most important sources and to identify potential high exposure population groups.

The sub-objectives:

- Identify and assess adverse health effects of BHT, and evaluate whether new studies indicate that the ADI established by EFSA needs to be revised
- Evaluate the scientific evidence on adverse health effects through a weight of evidence (WoE) approach
- Evaluate the quality of the data used for exposure assessment
- Estimate the exposure to BHT from specific sources using both deterministic and probabilistic methodology, and identify and describe the uncertainty related to the results
- Identify the most important BHT sources and exposure pathways
- Characterise the risk

2.2 Target age groups

The target age groups are

- Children (>1 - ⩽3 years)
- Children (>3 - ≤14 years)
- Adolescents (>14 - <18 years)
- Adults (≥18 years)

2.3 Chemical of concern

Description of BHT:

- Chemical name: butylated hydroxytoluene (BHT)
- Synonyms: 2,6-Bis(1,1-dimethylethyl)-4-methylphenol; 2,6-Di-t-butyl-p-cresol; 2,6-Bis(1,1-dimethylethyl)-4-methylphenol; Ionol; 1-Hydroxy-4-methyl-2,6-di-tert-butylbenzene; 2,6-Di-t-butyl-4-methylphenol; 2,6-Di-t-butyl-p-cresol; 2,6-Di-terc.butyl-p-kresol (Czech); 2,6-Di-tert-butyl-1-hydroxy-4-methylbenzene; 2,6-Di-tert-butyl-4-cresol; 2,6-Di-tert-butyl-4-hydroxytoluene; 2,6-Di-tert-butyl-4-methylhydroxybenzene; 2,6-Di-tert-butyl-4-methylphenol; 2,6-Di-tert-butyl-p-cresol; 2,6-Di-tert-butyl-p-methylphenol; 3,5-Di-tert-butyl-4-hydroxytoluene; 4-Hydroxy-3,5-

di-tert-butyltoluene; 4-Methyl-2,6-di-tert. butylfenol (Czech); 4-Methyl-2,6-di-tert-butylphenol; 4-Methyl-2,6-tert-butylphenol; Alkofen BP; Antioxidant 264; Antioxidant 29; Antioxidant 30; Antioxidant 4; Antioxidant 4K; Antioxidant DBPC; Antioxidant KB; Antox QT; Butylated hydroxytoluol; Butylhydroxytoluene; Butylohydroksytoluenu (Polish); Di-tert-butyl-p-cresol; Di-tert-butyl-p-methylphenol; Dibunol; Dibutylated hydroxytoluene; Impruvol; Stavox; Tonarol; Vulkanox KB; o-Di-tert-butyl-p-methylphenol;

- INCI name: BHT
- CAS number: 128-37-0
- EINECS number: 204-881-4
- EC Number: 204-881-4
- Molecular formula: $C_{15}H_{24}O$
- Molecular weight: 220.35

2.4 Literature searches and eligibility criteria for study selection

Separate literature searches will be performed to identify publications useful for answering the hazard identification and characterisation and exposure assessment sub-questions. An information specialist will conduct the literature searches.

The literature searches will be conducted in the following bibliographic databases:

- Ovid MEDLINE®
- Embase
- ISI Web of Science
- Scopus
- Cochrane Database of Systematic Reviews
- Epistemonikos
- SciFinder (American Chemical Society)

Less than 1% of the pharmaceuticals on the Norwegian market contain BHT (personal communication, the Norwegian Medicines Agency, September 4, 2017), and will contribute negligibly to the exposure of BHT. Therefore, exposure calculations for BHT from pharmaceuticals will not be performed, and studies reporting exclusively on BHT in pharmaceuticals are excluded.

The search strategy aims to retrieve studies on adverse health effects of BHT and studies reporting analytical data on BHT from the relevant sources

Different inclusion and exclusion criteria for study selection will be used to retrieve publications useful for the hazard identification and characterisation and the exposure assessment, respectively.

The evidence retrieved from each bibliographic database will be imported and combined in the bibliographic reference management software EndNote. Reviews will only be used to

check whether they contain additional references of primary studies that have not been captured by the literature searches.

2.5 Methods for selection of studies

A step-wise procedure is foreseen, as follows:

1. ***Screening of titles and abstracts:*** The screening of titles and abstracts will be performed by two reviewers working independently. When in doubt about inclusion, the paper will be considered as meeting the inclusion criteria.
2. ***Screening of full-text documents:*** For records passing the first screening based on titles and abstracts, the full text will undergo a second screening against the inclusion criteria by means of two reviewers working independently.

In case of disagreement, the two reviewers will discuss the paper in order to reach consensus. If the disagreement persists, the article will be brought to the attention of the Panel for discussion and agreement on a final decision.

The results of the different steps of the study selection process will be reported separately for exposure assessment and hazard identification and characterisation, and will be presented in the final assessment in flowcharts.

2.6 Data extraction from included studies

Pre-defined data extraction forms (modified from EFSA et al. (2017)) will be used to collect the data from the studies included in the assessment. Data extraction will be performed by one reviewer and checked for quality/consistency by a second reviewer.

3 Hazard identification and characterisation

3.1 Sub-questions to be answered in the hazard identification and characterisation

The sub-questions to be answered in the hazard identification and characterisation and the review approach, is presented in Table 3.1-1. A full systematic procedure will be applied to identify studies reporting on adverse health effects in humans and/or animals. For studies on genotoxicity and toxicokinetics, the approach is narrative.

Table 3.1-1. Sub-questions to be answered in the hazard identification and characterisation.

Risk assessment step	No.	Sub-questions to be answered in the hazard identification and characterisation	Approach
Hazard identification	1	Is exposure to BHT related to adverse health effects in humans? Identify target organs	Systematic
Hazard identification	2	Is exposure to BHT related to adverse health effects in animals? Identify target organs	Systematic
Hazard identification	3	Is BHT associated with changes at the molecular level such as mutation and other genotoxicity endpoints?	Narrative
Hazard characterisation	4	What is the nature of any dose-response relationship between BHT and relevant endpoints in the target organs in human and/or animal studies?	Systematic
Hazard characterisation	5	What is the ADME* in humans and in different animal species/strains?	Narrative
Hazard characterisation	6	Is there a difference in ADME between humans and animals?	Narrative
Hazard characterisation	7	Are the included human/animal studies biased according to the defined criteria?	Risk of bias evaluation

*ADME - absorption, distribution, metabolism, excretion

3.2 Literature search and endpoints relevant to the hazard identification and characterisation

A literature search will be performed to retrieve studies on adverse health effects of BHT. In 2012, EFSA established an ADI of 0.25 mg/kg bw per day (EFSA, 2012), based on a NOAEL of 25 mg/kg bw per day derived from two 2-generation studies and an uncertainty factor of 100.

In the present assessment, we will evaluate whether new studies exist that indicate that the ADI established by EFSA needs to be revised. Therefore, the search period will be limited to the time period from 2012 to present, and only studies that may result in a revised ADI will be included for the hazard identification and characterisation. The relevant endpoints for BHT are genotoxicity, chronic toxicity and carcinogenicity, reproductive and developmental effects, and neurological, neurobehavioral and neuroendocrine effects. If the ADI is not revised, the ADI established by EFSA will be used for the risk characterisation (EFSA, 2012).

3.3 Methods for gathering evidence

For human studies and animal studies, identified using a systematic approach, data will be collected using data extraction forms, and risk of bias will be evaluated.

3.3.1 Inclusion/exclusion criteria for hazard identification and characterisation

A short description of the literature search is given in Chapter 2.4.

Tables 3.3.1-1, 3.3.1-2 and 3.3.1-3 list the criteria for including or excluding human, animal and *in vitro* studies in the hazard identification and characterisation, respectively. For *in vitro studies*, only studies addressing genotoxicity will be included in the hazard identification and characterisation.

Table 3.3.1-1. Inclusion/exclusion criteria for human studies in the hazard identification and characterisation.

Literature screening for data related to the following sub-questions to be answered in the hazard identification and characterisation **1: Is exposure to BHT related to adverse health effects in humans? Identify target organs.** **4: What is the nature of any dose–response relationship between BHT and relevant endpoints in the target organs in human studies?** **5: What is the ADME* in humans?** **6: Is there a difference in ADME between humans and animals?**		
Study design	In	Human studies, including cohort studies, case-control studies (prospective, retrospective and nested), and toxicokinetic biomonitoring studies on any route of exposure
	Out	Animal studies and *in vitro/in silico* studies
Population	In	Children (>1 - ≤14 years), adolescents (>14-<18 years) and adults (≥18 years)
	Out	Infants
Exposure	In	All routes of exposure
	Out	
	In	All reported adverse health effects

Literature screening for data related to the following sub-questions to be answered in the hazard identification and characterisation **1: Is exposure to BHT related to adverse health effects in humans? Identify target organs.** **4: What is the nature of any dose–response relationship between BHT and relevant endpoints in the target organs in human studies?** **5: What is the ADME* in humans?** **6: Is there a difference in ADME between humans and animals?**		
Outcome of interest	Out	Studies reporting exclusively preventive/beneficial effects on the target organs, and studies reporting exclusively on the antioxidant properties/activities of BHT
Language of the full text	In	English, Norwegian, Swedish, Danish, German
Publication type	In	E.g. primary research studies, systematic reviews, meta-analyses and risk assessments
	Out	Editorials Letters to the editor Book chapters Meeting's abstracts and posters

*ADME - absorption, distribution, metabolism, excretion

Table 3.3.1-2 Inclusion/exclusion criteria for animal studies in the hazard identification and characterisation.

Literature screening for data related to the following sub-questions to be answered in the hazard identification and characterisation **2: Is exposure to BHT related to adverse health effects in animals? Identify target organs.** **4: What is the nature of any dose-response relationship between BHT and relevant endpoints in the target organs in animal studies?** **5: What is the ADME* in different animal species/strains?** **6: Is there a difference in ADME between humans and animals?**		
Study design	In	*In vivo* studies on animals not examining genotoxicity. Toxicokinetic studies (narrative approach)
	Out	Human studies and *in vitro/in silico* studies
Population	In	All mammalian animals
	Out	Non-mammalian animals
Exposure	In	All routes of exposure
	Out	Studies where BHT is a part of a mixture and not tested alone.
Outcome of interest	In	All reported adverse health effects excluding genotoxicity
	Out	Studies reporting exclusively on the antioxidant properties/activities of BHT or studies on genotoxicity
Language of the full text	In	English, Norwegian, Swedish, Danish, German

Literature screening for data related to the following sub-questions to be answered in the hazard identification and characterisation **2: Is exposure to BHT related to adverse health effects in animals? Identify target organs.** **4: What is the nature of any dose-response relationship between BHT and relevant endpoints in the target organs in animal studies?** **5: What is the ADME* in different animal species/strains?** **6: Is there a difference in ADME between humans and animals?**		
Publication type	In	E.g. primary research studies, systematic reviews, meta-analyses and risk assessments
	Out	Editorials Letters to the editor Book chapters Meeting's abstracts and posters

*ADME - absorption, distribution, metabolism, excretion

Table 3.3.1-3. Inclusion/exclusion criteria for studies on genotoxicity.

Literature screening for data related to the following sub-question to be answered in the hazard identification and characterisation **3: Is BHT associated with changes at the molecular level such as mutation and other genotoxicity endpoints?**		
Study design/test systems	In	*In vitro* studies on genotoxicity *In vivo* studies on genotoxicity
	Out	Test systems: Drosophila melanogaster, Vicia faba, Allium cepa, fish Non-genotoxicity studies
Exposure	In	Route of exposure for animal *in vivo* studies: oral, subcutaneous, intraperitoneal All *in vitro* genotoxicity studies
	Out	Intravenous
Outcome of interest	In	• Gene (point) mutation • Structural and numerical chromosomal aberrations • Micronuclei • Endoreduplication, polyploidy • Sister chromatid exchange (SCE) • Unscheduled DNA synthesis (UDS)/DNA repair • Cell transformation
Language of the full text	In	English, Norwegian, Swedish, Danish, German
Publication type	In	E.g. primary research studies, systematic reviews, meta-analyses and risk assessments
	Out	Editorials Letters to the editor Book chapters Meeting's abstracts and posters

3.3.2 **Data extraction and evaluation of risk of bias**

Data from the included human studies will be extracted using Table 3.3.2-1.

Table 3.3.2-1. Data extraction form for human studies (modified from EFSA et al. (2017)).

Study ID	**Reference:**
	Study name and acronym (if applicable):
	Total number of subjects:
	Health outcome category:
Funding	Funding source: Public/private:
Study design	Study type:
	Type of blinding:
	Method for randomization:
	Year the study was conducted (start):
	Duration/length of follow-up:
	Dates of sampling (when relevant):
	Dates for analyses of BHT-conjugates in sample:
Subjects	Number of participants in the study:
	Participation rate:
	Number of subjects with measured levels:
	Number of exposed/non-exposed subjects or number of cases/controls:
	Follow-up rates by group (%):
	Sex (male/female):
	Geography (country, region, state, etc.):
	Age at exposure:
	Ethnicity:
	Socioeconomic background:
	Confounders and other variables as reported:
	Outcome assessment (e.g. mean, median, measures of variance as presented in paper such as standard deviation, standard error of the mean, 75^{th}/90^{th}/95^{th} percentile, minimum/maximum):
	Inclusion and exclusion criteria:
Intervention/exposure	Measured levels in human biological samples (e.g. breast milk, blood, and urine) and method used (validation of the method, measures to avoid contamination of samples, etc.):
	Estimated dietary exposure and method used (validation of the method, measures to avoid contamination of samples, limit of quantification and limit of detection etc.):
Methods for endpoint assessment	Parameters measured (units of measure, measures of central tendency and dispersion, confidence interval):
	Diagnostic or method to measure health outcome (including self-reporting):
Statistical analysis	Statistical methods :

Study ID	**Reference:**
	Study name and acronym (if applicable):
	Total number of subjects:
	Health outcome category:
Results	Measures of effect and all statistics at each exposure level as reported in the paper, and for each sub-group and end-point when applicable:
	Were sub-groups analyses predefined (yes/no, including justification):
	How the variables were treated (continuous, transformed, or categorical):
	Statistical test used, modifying factors and other potential sources of bias:
Other comments	

Data from the included animal studies will be extracted using Table 3.3.2-2.

Table 3.3.2-2. Data extraction form for animal studies (modified from EFSA et al. (2017)).

Study ID	**Reference:**
	Year the study was conducted (start, if available):
	Health outcome category:
Funding	Funding source: Public/private:
Type of study and guideline	Good laboratory practice (yes/no): Guideline studies (if yes, specify):
	Type of study:
Animal model	Species/(sub-)strain/line:
	Disease models (e.g. infection, diabetes, allergy, obesity):
Housing condition	Housing condition (including cages, bottles, bedding):
	Diet name and source:
	Background levels of phytoestrogens in the diet (type and levels):
Exposure	BHT provider:
	Compound purity:
	Vehicle used: Dose regimen (dose level or concentration of BHT per group, and frequency):
	Route of administration (diet, drinking water, gavage, subcutaneous, intraperitoneal, dermal, inhalation):
	Period of exposure (pre-mating, mating, gestation, lactation, adult):
	Duration of the exposure:
Study design	Sex and age of the initially exposed animals:
	Number of groups/number of animals per group:
	Randomisation procedures at start of the study:
	Reducing (culling) of litters and method:
	Number of pups per litter for next generation and methodology:
	Number of pups per litter/animals for certain measurements and methodology:
	Time of measurement/Observation period (premating, mating, gestation, lactation, adult):

Study ID	**Reference:**
	Year the study was conducted (start, if available):
	Health outcome category:
	Endpoints measured:
	Methods to measure endpoints:
	Estimated dietary exposure and method used (validation of the method, measures to avoid contamination of samples, limit of quantification and limit of detection etc.)
Statistical analysis	Statistical methods:
Results	Concentration of the test compound in vehicle (analysed, stated, ambigous):
	Documentation of details for dose conversion when conducted:
	Level of test compound in tissue or blood:
	Results per dose or concentration (e.g. mean, median, frequency, measures of precision or variance):
	No observed adverse effect level, lowest observed adverse effect level, benchmark dose/benchmark dose lower bound, and statistical significance of other dose levels (author's interpretation):
	Shape of dose response if reported by the authors:
Other comments	

In this assessment, the evaluation of risk of bias includes consideration of two aspects:

- Aspects that introduce a systematic difference between the control and the exposed group only (e.g. non-randomised allocation of animals to study groups)
- Aspects potentially affecting, to the same extent, control and exposed study groups (e.g. the reliability of the method used to test the outcome).

The questions addressed to assess the risk of bias in the human and animal studies are presented in Table 3.3.2-3 and Table 3.3.2-4, respectively (NTP, 2015). For each question in Table 3.3.2-3 and Table 3.3.2-4, the response options are "Definitely low risk of bias (++)", "Probably low risk of bias (+)", "Probably high risk of bias (-)", "Definitely high risk of bias (--)" (Table 3.3.2-5). Whenever an element to be evaluated is not reported, this will by default be judged as "Probably high risk of bias".

Table 3.3.2-3. Evaluation of risk of bias in human studies (modified from EFSA et al. (2017)).

Number	Question	Domain	Rating (++, +, -, --)
1	Did selection of study participants result in appropriate comparison groups?	Selection	
2	Can we be confident in the exposure characterisation?	Detection	
3	Can we be confident in the outcome assessment?	Detection	

Number	Question	Domain	Rating (++, +, -, --)
4	Did the study design or analysis account for important confounding and modifying variables?	Confounding	
5	Do the statistical methods seem appropriate?	Other sources of bias	

Table 3.3.2-4. Evaluation of risk of bias in animal studies (modified from EFSA et al. (2017)).

Number	Question	Domain	Rating (++, +, -, --)
1	Were experimental conditions identical across study groups?	Performance	
2	Were outcome data completely reported without attrition or exclusion from analysis?	Attrition	
3	Can we be confident in the exposure characterisation?	Detection	
4	Can we be confident in the outcome assessment?	Detection	
5	Were the statistical methods and the number of animals per dose group appropriate?	Other sources of bias	

Table 3.3.2-5. Response options for evaluation of risk of bias (modified from EFSA et al. (2017)).

Rating	Response to the question	Description
++	**Definitely low** risk of bias	There is direct evidence of low risk of bias practices
+	**Probably low** risk of bias	There is indirect evidence of low risk of bias practices, or it is deemed that deviations from low risk of bias practices for these criteria during the study would not appreciably bias results. This includes consideration of direction and magnitude of bias
-/not reported	**Probably high** risk of bias	There is indirect evidence of high risk of bias practices, or there is insufficient information provided about the relevant risk of bias practices
--	**Definitely high** risk of bias	There is direct evidence of high risk of bias practices

The ratings of the questions (++, +, -, --) will be integrated to classify the studies in tiers from 1 to 4 corresponding to decreasing levels of risk of bias. Two reviewers will perform

each evaluation independently. In case of disagreement, the reviewers will discuss until consensus is reached or the VKM Panel will reach a final decision.

3.4 Evaluation of relevance of the endpoints for the target population

For the animal studies, the relevance of the specific endpoints studied for the human target population will be evaluated. The evaluation will be performed by two reviewers independently. In case of disagreement, the reviewers will discuss until consensus is reached or the VKM Panel will reach a final decision.

3.5 Weighting the body of evidence

All studies reporting on a given endpoint will be grouped, and the evidence will be weighted using a modified version from (EFSA et al., 2017), downgrading or upgrading the confidence in the evidence. Several elements will be considered for downgrading or upgrading the confidence in the evidence:

Elements that may cause downgrading of the confidence in the evidence are:

- Risk of bias
- Relevance of endpoints (for animal studies only)
- Unexplained inconsistency
- Imprecision

Elements that may cause upgrading of the confidence in the evidence are:

- Large effect (e.g. incidence, degrees of severity)
- Dose-response relationship
- Consistency, across study design type, dissimilar populations, animal models, species or gender
- Consistency in direction of effect
- Confounding, if all relevant confounders are described and taken into account

Table 3.5.-1 will be used for the downgrading/upgrading of the evidence. One table will be used per endpoint. After the downgrading/upgrading of the evidence, the terms used for the overall confidence in the evidence are:

- **High confidence (++++)** in the association between exposure to the substance and the outcome. The true effect is highly likely to be reflected in the apparent relationship.
- **Moderate confidence (+++)** in the association between exposure to the substance and the outcome. The true effect may be reflected in the apparent relationship.
- **Low confidence (++)** in the association between exposure to the substance and the outcome. The true effect may be different from the apparent relationship.

- **Very low confidence (+)** in the association between exposure to the substance and the outcome. The true effect is highly likely to be different from the apparent relationship.

Table 3.5-1. Grading confidence in the body of evidence per endpoint (modified from EFSA et al. (2017)).

Endpoint [describe]									
	Elements triggering downgrading				**Elements triggering upgrading**				Overall confidence level
Reference	Risk of bias	Relevance of endpoint (animal studies only)	Unexplained inconsistency	Imprecision	Large effect	Dose-response relationship	Consistency	Confounding	
Reference 1	Describe identified risks	Discuss use of endpoints or models with less relevance to humans	Describe results in terms of consistency Explain apparent inconsistency (if it can be explained)	Discuss ability to distinguish treatment from control Describe confidence intervals	Describe magnitude of response	Outline evidence for or against dose response	Describe cross-species, model, or population consistency	Address whether there is evidence that confounding would bias toward null	Confidence level
Reference 2									
Reference 3									
Overall conclusion on confidence									Chosen confidence interval

To decide if each endpoint represents an adverse or no adverse health effect will be based on the overall confidence in the body of evidence. The impact of new evidence on the ADI established by EFSA will be evaluated (EFSA, 2012).

It is important to emphasize that the likelihood assessed by the WoE approach refers specifically to hazard identification, i.e. it refers to the likelihood of an association between BHT and the effect under consideration. It does *not* refer to the likelihood or frequency of the effect actually occurring in humans, which depends on additional factors including the dose-response relationship for the effect (considered in hazard characterisation) and the levels of human exposure to BHT (considered in exposure assessment).

3.6 Method for performing hazard characterisation

For the hazard characterisation, the overall confidence in the evidence of each endpoint is transformed to likelihood (Table 3.6-1).

Table 3.6-1. Set of terms used to transform the overall confidence "interval" in the evidence per endpoint to overall likelihood.

Likelihood of an association between BHT and the effect under consideration	Summary confidence range levels
Very likely	++++
Likely	From ++++ to +++
As likely as not	From +++ to ++
Unlikely	From ++ to +
Very unlikely	+

Dose-response analysis will be performed for "Very likely" and "Likely" effects, using human and/or experimental animal studies showing adverse health effects relevant to humans. An effect is considered "adverse" when leading to "change in the morphology, physiology, growth, development, reproduction or lifespan of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences" (WHO, 2009). Given the broad number of endpoints examined, the adversity of a specific effect and the critical effect size will be evaluated case-by-case based on expert judgement. A justification will be provided.

For the hazard characterisation, it is intended to include the analysis of the dose-response relationship and the identification of a reference point (benchmark dose (BMD) and its lower confidence limit (BMDL) for a particular incidence/size of effect) as a basis for a new ADI. Analysis of the data will be performed according to the EFSA Guidance on the use of the BMD approach in risk assessment (EFSA et al., 2017).

3.7 Uncertainty in hazard identification and characterisation

The uncertainty evaluation of hazard identification and characterisation will be described qualitatively, and an overview is given in Table 3.7-1.

Table 3.7-1. Qualitative evaluation of influences of uncertainties on the hazard identification and characterisation.

Endpoint	Source of uncertainty	Direction

+: uncertainty likely to cause over-estimation of the hazard
-: uncertainty likely to cause under-estimation of the hazard

4 Exposure

First, concentrations of BHT in foods, cosmetics, indoor dust and indoor air will be identified and compiled in a database. Next, BHT exposure will be estimated using this database and data on consumption/inhalation. The national food consumption surveys provide data on individual food consumption. The human biomonitoring study EuroMix will provide individual data on both food consumption and use of cosmetics. None of the studies has measures on indoor dust or indoor air, therefore calculations will rely on default values for consumption/inhalation and BHT levels from the database compiled for this assessment.

External exposure - BHT reaching the physical barriers in the body; internal exposure - absorbed BHT; and aggregated exposure - toxic BHT (BHT and/or toxic metabolites), will be estimated. Both deterministic and probabilistic methodology will be used.

The uncertainty related to the results using deterministic and probabilistic methodology will be identified and estimated.

4.1 Sources and routes of exposure

Chronic BHT exposure from foods, cosmetics, indoor dust and indoor air, will be estimated. Foods include all foods and all drinks. Cosmetics includes cosmetics and personal care products.

The routes of exposure are oral, dermal and inhalation.

4.2 Sub-questions to be answered in the exposure assessment

An overview of the sub-questions to be answered in the exposure assessment is given in Table 4.2-1.

Table 4.2-1. Exposure assessment sub-questions.

Risk assessment step	No.	Sub-questions to be answered in the exposure assessment	Approach
Exposure assessment	1	What are the exposure levels and sources of BHT from foods?	Systematic
Exposure assessment	2	What are the exposure levels and sources of BHT from cosmetics?	Systematic
Exposure assessment	3	What are the exposure levels and sources of BHT from indoor dust?	Systematic
Exposure assessment	4	What are the exposure levels and sources of BHT from indoor air?	Systematic
Exposure assessment	5	What is the aggregated exposure to BHT?	Systematic

4.3 Literature search

A literature search will be performed to retrieve studies reporting concentrations of BHT in foods, cosmetics, indoor dust and indoor air.

4.4 Method for gathering evidence

4.4.1 Inclusion/exclusion criteria for exposure assessment study selection

The literature search is described in Chapter 2.4. The inclusion and exclusion criteria for the study selection are listed in Table 4.4.1-1.

Table 4.4.1-1. Inclusion/exclusion criteria for the exposure assessment study selection.

Literature screening for concentration data related to the following sub-questions to be answered in the exposure assessment **1: What is the exposure to BHT from foods?** **2: What is the exposure to BHT from cosmetics?** **3: What is the exposure to BHT from indoor dust?** **4: What is the exposure to BHT from indoor air?**		
Study design	In	All publications that address analyses of BHT as concentrations, exposure and/or intake
	Out	Human studies, animal studies, *in vitro* studies
Study characteristics	In	Studies presenting analytical data and biomonitoring data on BHT
	Out	-
Analytical method	In	All methods
	Out	-
Sources and outcome of interest	In	BHT concentrations in foods, cosmetics, indoor dust and indoor air
	Out	Studies reporting exclusively on toxicity or preventive/beneficial effects Studies reporting exclusively on the antioxidant properties/activities Studies reporting exclusively on BHT in pharmaceuticals or other sources
Language of the full text	In	English, Norwegian, Swedish, Danish, German
Publication type	In	Primary research articles Risk assessments and reports
	Out	Editorials Letters to the editor Book chapters Meeting's abstracts and posters

4.4.2 Data extraction

Data from the included studies will be extracted using Table 4.4.2-1.

Table 4.4.2-1: Data extraction form for BHT concentration data (modified from EFSA et al. (2017)).

Study ID	**Reference:**
	Year the study was conducted/published:
	Source category:
Funding	Funding source: Public/private:
Aim of the study	Analysis:
	Exposure:
	Migration:
Concentration in foods/non-foods	Food:
	Cosmetics:
	Indoor dust:
	Indoor air:
Methods for analysis	Sample extraction:
	Calibration:
	Limit of detection/limit of quantification:
	Recovery data:
	Instrument/detector:
Results	Number of samples:
	Concentration:
Other comments	e.g. risk of contamination

For all included studies, the data quality will be evaluated. An overview of the questions asked for the evaluation of data quality is given in Table 4.2.2-2. This evaluation includes scoring of the sample extraction method, the instrumental analysis, and the validation of the method and the data presentation. The core will be deduced according to a scale of scores from 1 (lowest quality) to 5 (highest quality). To obtain the total score, the individual scores are weighted: 1/5 from sample extraction, 1/5 from instrumental analysis, and 3/5 from validation and data presentation. Only articles with a total score of ≥ 3,5 will be used for the exposure assessment.

Table 4.4.2-2: Form for evaluation of data quality.

No.	Question	Rating (1-5)
1	How appropriate was the solvent used for the extraction method (diethyl ether = hexane > EtOH > MeOH not H_2O)?	
2	Which instrumental analysis was used (e.g. GC-MS/GC-FID and HPLC-UVD, LC-MS)?	
3	Which validation method has been used, and how has the data been presented (LOD/LOQ, internal/external calibration, number of samples, statistical methods)?	
	Total score (1/5 x sample extraction+1/5 x instrumental analysis+3/5 x validation and data presentation)	

4.5 Exposure estimation – scenarios and methods

The population is exposed to BHT from e.g. foods and/or cosmetics and/or indoor dust and/or indoor air. The exposure pathways may be oral and/or dermal and/or through inhalation.

The exposure will be estimated for:

- BHT reaching the physical barriers in the body – here defined as the external exposure. External exposure will be estimated separately for foods, cosmetics, indoor dust and indoor air
- Absorbed BHT – here defined as the internal exposure. The internal exposure from all sources will be given as one number, and this can be compared with biomonitoring data
- Toxic BHT (BHT and/or toxic metabolites) – here defined as the aggregated exposure. The aggregated exposure will be compared with the ADI

An overview of the external, internal and aggregated exposure is given in Figure 4.5-1.

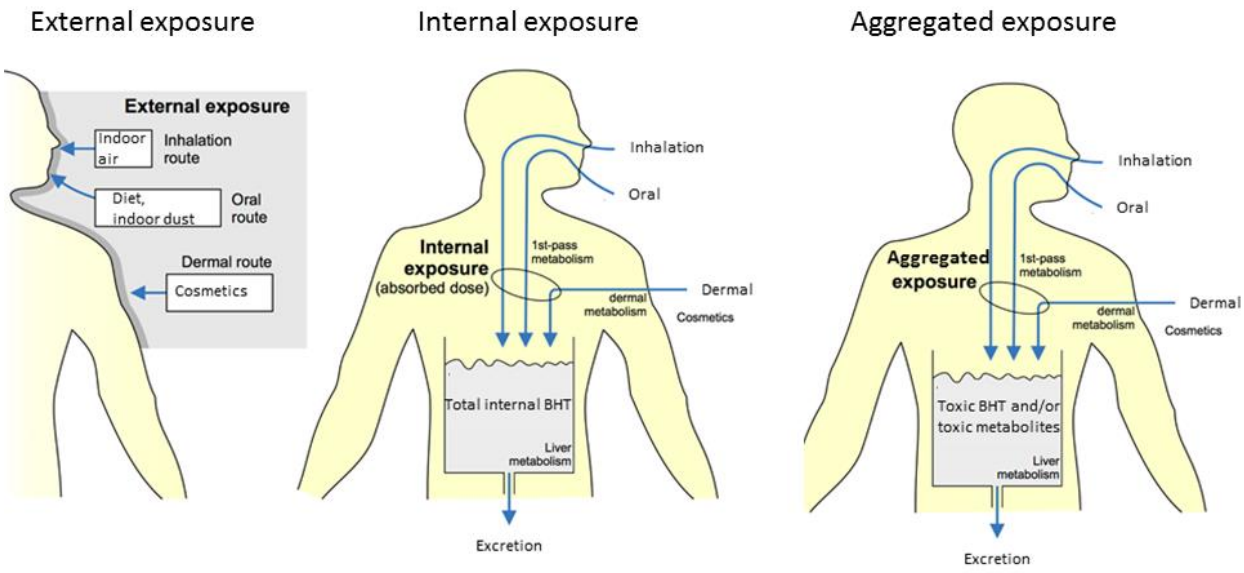

Figure 4.5-1. External exposure, internal exposure (i.e. absorbed dose) and aggregated exposure (modified from EFSA (2015)).

For the exposure assessment, a detailed concentration database including data on BHT concentrations in foods, cosmetics, indoor dust and indoor air will be developed based on available data in articles and previous risk assessments identified in the literature search.

Left-censored data, i.e. data from samples with concentrations below the limit of detection (LOD) or limit of quantification (LOQ), will be handled through the substitution method. The lower bound (LB) will be obtained by assigning a value of zero to all the samples reported as less than the left-censoring limit, the middle bound (MB) by assigning half of the left-

censoring limit, and the upper bound (UB) by assigning the left-censored limit (LOD or LOQ) as the sample value result.

The exposure assessment will be performed both deterministically and probabilistically for all age groups, depending on available data. However, lack of data may add limitations for the assessment of some age groups.

The deterministic approach will use single values and simple scenarios to produce a point estimate of typical exposure and high-end exposure. The deterministic estimates use readily available data, and produce results that are straightforward to interpret.

Probabilistic estimates rely on distributions as inputs in place of single values for key parameters. This results in a distribution of possible exposure estimates and greater ability to characterise variability and uncertainty. The Monte Carlo Risk Assessment tool (MCRA, developed by the National Institute for Public Health and the Environment (RIVM) in the Netherlands)) will be used for the probabilistic exposure assessment. This is a web-based system for probabilistic modelling of exposure to chemicals in the diet. Additionally, custom made scripts in the software *R* will be developed to perform both deterministic and probabilistic calculations of exposure.

4.5.1 Estimation of BHT reaching the physical barriers in the body – external exposure

BHT from different sources and exposure pathways that reaches the physical barriers in the body, the external exposure, will be estimated. The assessment of external exposure includes BHT reaching the gastrointestinal tract (oral intake of foods and dust), the respiratory tract (inhalation of BHT in indoor air) and the skin (dermal exposure to BHT in cosmetics).

For dietary exposure estimation, food consumption data sets and concentration data for BHT in foods will be used. Food consumption data sets include the Norwegian food consumption surveys (for all age groups) and the food consumption data from the human biomonitoring study EuroMix (adults). Concentration data for BHT in foods are retrieved from the BHT concentration database compiled for this project. These data sets have different levels of detail and representativeness, and calculations will be performed on each data set independently.

The estimation of BHT exposure from cosmetics will be based on concentration data for BHT in cosmetics and personal care products. Concentration data for BHT in cosmetics is retrieved from the concentration database compiled for this project. Use of cosmetics (frequencies) is available from the EuroMix study.

The estimation of BHT exposure from indoor dust will be based on mean oral dust intake from relevant literature, combined with concentration data for BHT in indoor dust. Concentration data for BHT in indoor dust is retrieved from the BHT concentration database compiled for this project.

The estimation of BHT exposure from indoor air will be based on estimated breathing volumes and anticipated time spent indoors from relevant literature, combined with concentration data for volatile BHT in air. Concentration data for BHT in indoor air is retrieved from the BHT concentration database compiled for this project.

Aggregated exposure to BHT from several sources and exposure pathways will be estimated, and the relative contribution from the different sources will be calculated.

4.5.2 Estimation of absorbed BHT – internal exposure

Absorbed BHT, the BHT that passes the gastrointestinal tract, the respiratory tract and the skin, is defined as the internal exposure. The internal exposure of BHT includes uptake via all routes, and is directly comparable to urinary biomonitoring data. The absorption fractions for the different routes of exposure are taken into account when estimating the internal exposure.

The internal exposure to BHT ($T_{internal}$) will be estimated by summing the products of the absorption factor (alfa) and external exposure (T) for each exposure source as follows:

$T_{internal} =$	Total internal exposure [mg / kg body weight]
$\alpha_F \times T_F$	absorption factor for food (α_F)× external exposure from food (T_F)
$+\alpha_C \times T_C$	absorption factor for cosmetics (α_C)× external exposure from cosmetics (T_C)
$+\alpha_D \times T_D$	absorption factor for dust (α_D) × external exposure from dust (T_D)
$+\alpha_A \times T_A$	absorption factor for air (α_A) × external exposure from air (T_A)

F= food; C=cosmetics, D=dust; A=air

4.5.3 Internal exposure to toxic substances (BHT and/or its metabolites) – aggregated exposure

Potential toxic substances, including BHT and its metabolite(s), will be qualitatively assessed pending the outcome of the ADME (see 3.3). If data and parameters (metabolic rates, toxic equivalency factors etc.) are identified that enable calculation of aggregated exposure to all toxic substances, such calculation will be performed.

4.5.4 **Exposure estimation using a deterministic approach**

4.5.4.1 External exposure

For foods, exposure will be estimated based on data for mean concentration of BHT per foods, and consumption of the foods. We will obtain exposure estimates (mean/median and high) for each food consumption data set.

For cosmetics, exposure will be estimated based on data for mean concentrations of BHT in cosmetic products and frequency of use. The frequencies of use will be adjusted for the exposure fraction, that is the fraction (between 1 and 0) of the cosmetic product that will stay on the skin after application and is available for dermal absorption. We will obtain exposure estimates (mean/median and high) from the EuroMix study.

For indoor dust, exposure will be estimated based on data for mean concentration of BHT in indoor dust, and oral intake of dust. We will obtain exposure estimates (mean/median and high) using regularly used default values for intake.

For indoor air, exposure will be estimated based on data for mean concentration of BHT in indoor air, and inhalation of indoor air based on time spent indoors. We will obtain exposure estimates (mean/median and high) using regularly used default values for intake.

In addition, the EuroMix study will allow for a combined exposure through diet and cosmetics, since consumption data for the same individuals for both routes are available. This will allow for proper inclusion of covariation between diet and use of cosmetics. Combined exposure estimates (mean/median and high) including diet and cosmetics will be obtained from the EuroMix study.

4.5.4.2 Internal exposure

To estimate internal exposure to BHT, the external exposure (estimated in 4.5.4.1) is multiplied with the absorption factors for different exposure pathways.

Mean exposure from different sources will be added together to assess mean total internal exposure. High total internal exposure will be assessed by adding the two highest values from the exposure sources with the mean exposure value from the two other sources.

4.5.4.3 Aggregated exposure

For estimation of aggregated exposure, different combinations of mean and high estimates of BHT or its toxic metabolite(s) are explored. Mean exposure from different sources will be added together to assess mean total aggregated exposure. High total aggregated exposure will be assessed by adding the two highest exposure sources with the mean exposure from the other two sources.

4.5.5 **Exposure estimation using a probabilistic approach**

External and internal exposure (see 4.5) will also be calculated using probabilistic methods. In general, these methods allow for better control, inclusion and quantification of uncertainties in exposure assessments, including measurement uncertainty, variability in concentrations for different foods and cosmetics, consumption/application rates across individuals and allow for a better incorporation of multiple routes of exposure. Probabilistic exposure estimates will be performed using two different implementations; MCRA software and custom-made scripts in the *R* statistical software. MCRA is developed to include various sources of variability in assessing exposure through diet, and allows for inclusion of individual level uptake through other routes, though with less ability to include uncertainty and variability in non-dietary routes.

4.6 Uncertainty in the exposure assessment

4.6.1 **Uncertainty in the deterministic exposure assessment**

The evaluation of uncertainties in the deterministic exposure assessment is based on expert judgement.

The expert knowledge elicitation on the uncertainties in the deterministic exposure assessment will take place before results from the probabilistic exposure are presented.

Every part of the exposure assessment will be systematically examined for potential sources of uncertainty. The identified uncertainties will be listed in a table, the impact of each uncertainty on the outcome of the exposure assessment will be evaluated by expert elicitation, and the combined impact of all the uncertainties on the outcome of the exposure assessment will be evaluated.

The impact on the exposure estimate is graded as shown in Figure 4.6.1.1-1.

- Plus symbols (+) indicate that the true value of the exposure could be higher than the estimate.
- Minus symbols (-) indicate that the true value could be lower than the estimate.
- Dot (●) means that the impact of the uncertainty is less than +/– 20%, either higher or lower.

As the evaluation is approximate, each symbol represents a range of possible values; for example, "++" means that the true exposure is judged to be between two and five times the estimate. Pairs of symbols are used where the uncertainty spans a larger range; for example "–/++" would mean that the true value exposure is judged to be between half and five times the estimate. However, the relative likelihood of different values within the range was not assessed.

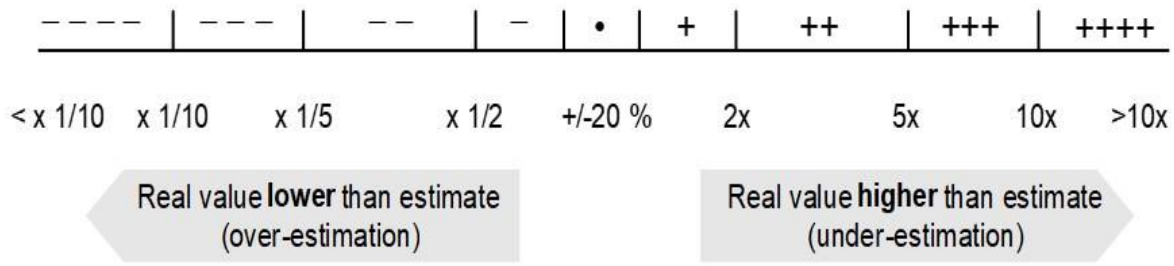

Figure 4.6.1.1-1. The scale used to grade the impact of uncertainties on the exposure estimates (modified from EFSA (2015)).

An overview of the deterministic uncertainty evaluation, including the source of uncertainty and the estimated impact on the exposure estimate, is given in Table 4.6.1.1-1.

Table 4.6.1.1-1. An overview of the deterministic uncertainty evaluation and the estimated impact on the exposure estimate.

Source of uncertainty	Factor effected	Value used in assessment	Impact on exposure estimate
Foods:			
Cosmetics:			
Indoor dust:			
Indoor air:			
Overall assessment			

4.6.2 **Uncertainty in the probabilistic exposure assessment**

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5 Risk characterisation

In the risk characterisation

- The aggregated exposure will be compared with the ADI
- The most important BHT sources (foods, cosmetics, indoor dust or indoor air) will be identified
- Potential high exposure population groups will be identified

6 References

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