Screening Assessment for the Challenge

Phenol, (1,1-dimethylethyl)-4-methoxy- (Butylated hydroxyanisole)

Chemical Abstracts Service Registry Number
25013-16-5

Environment Canada

Health Canada

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Synopsis

The Ministers of the Environment and of Health have conducted a screening assessment of phenol, (1,1-dimethylethyl)-4-methoxy- (also known as butylated hydroxyanisole or BHA), Chemical Abstracts Service Registry Number 25013-16-5. The substance BHA was identified in the categorization of the *Domestic Substances List* as a high priority for action under the Challenge, as it was determined to present intermediate potential for exposure of individuals in Canada and was considered to present a high hazard to human health, based upon classification by other agencies on the basis of carcinogenicity. The substance did not meet the ecological categorization criteria for persistence, bioaccumulation or inherent toxicity to aquatic organisms. Therefore, this assessment focuses principally upon information relevant to the evaluation of risks to human health.

According to information reported in response to a notice published under section 71 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), no BHA was manufactured in Canada in 2006 at quantities equal to or greater than the reporting threshold of 100 kg. However, between 100 and 1000 kg of BHA was imported into Canada, while between 1000 and 10 000 kg of BHA was used in Canada alone, in a product, in a mixture or in a manufactured item. BHA is permitted for use in Canada as an antioxidant in food. In fats and fat-containing foods, BHA delays the deterioration of flavours and odours and substantially increases the shelf life. It is appropriate for use in foods baked or fried in animal oils because of its high thermal stability and ability to remain active in foods cooked in this manner. BHA is a primary antioxidant used in animal feeds, as it retards the oxidation of vitamins A and E, carotene, xanthophyll pigments, rendered fats and vegetable oils. BHA is a non-medicinal ingredient used as an antioxidant and antimicrobial preservative in final pharmaceutical products, natural health products and veterinary products manufactured in Canada. It is a constituent of personal care products, such as deodorants, shampoos and body lotions, in Canada. BHA is a formulant in pesticide products in Canada. It is a stabilizer or fragrance in rodenticides, insecticides (home garden), fungicides, bactericides (pulp and paper), insecticidal shampoos (cats and dogs), insect repellents (lotion, towelettes) and antibacterial glass and surface cleaners. In Canada, the general population is exposed to BHA through its permitted use as an antioxidant in foods and through its use in personal care products such as shampoos and skin moisturizers.

Based principally on weight of evidence–based assessments of international and other national agencies, the critical effect for the characterization of risks to human health from exposure to BHA is carcinogenicity. Lifetime feeding of BHA at high concentrations induced tumours in the forestomach of the rat, mouse and hamster, while no tumours were reported in feeding studies in animals that do not have a forestomach (guinea pig, pig, dog or monkey). It has been postulated that BHA may not be a carcinogen in animals that lack a forestomach. Consideration of the available information regarding genotoxicity indicates that BHA is not likely to be genotoxic. Accordingly, although the mode of induction of tumours is not fully elucidated, the tumours observed are not considered to have resulted from direct interaction with genetic material. Therefore, a threshold approach is used to assess risk to human health.

Based on the comparison of estimated exposures to BHA in Canada with the critical effect levels, and taking into account the uncertainties in the databases on exposure and effects, it is considered that the resulting margins of exposure are adequately protective of human health for non-cancer effects. Margins of exposure for cancer effects are also considered adequate to protect human health. The margins between these levels of exposure and estimates of intake resulting from use of personal care products are considered to be adequately protective of human health.

Based on the available information on its potential to cause harm to human health, it is concluded that BHA is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Based on empirical data for an analogue (4-*tert*-butylphenol), and modelled data, it is concluded that BHA is likely to degrade quickly in air, water and soil but not in sediment. In addition, based on experimental data for an analogue (4-*tert*-butylphenol) as well as modelled data, it is concluded that BHA does not meet the bioaccumulation criteria.

As BHA can be found in consumer products and is reported to be released to water, a specific scenario was developed to calculate a risk quotient based on a comparison between the highest known municipal effluent concentration and the predicted no-effect concentration. Secondly, a scenario based upon down-the-drain releases from consumer uses was used to estimate the potential concentration of BHA in multiple water bodies receiving STP effluents. Thirdly, a site-specific ecological exposure scenario for five sites was developed based on the information on commercial use to conservatively estimate releases into the aquatic environment from industrial operations and resulting aquatic concentrations. Environmental concentrations were estimated to be below those that would harm sensitive aquatic organisms. These three scenarios indicate that the substance is unlikely to cause ecological harm in the aquatic environment.

On the basis of available information, including results from conservative risk quotient calculations, as well as information on persistence, bioaccumulation and toxicity of the substance, it is concluded that BHA 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 or its biological diversity or that constitute or may constitute a danger to the environment on which life depends. BHA meets the persistence criteria but does not meet the criteria for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations.*

Based on the information available, it is concluded that BHA does not meet the criteria set out in section 64 of CEPA 1999.

This substance will be considered for inclusion in the *Domestic Substances List* inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

Introduction

Under the *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999), the Minister of the Environment and the Minister of Health conduct screening assessments of substances that were prioritized during the categorization of substances on the *Domestic Substances List* to determine whether these substances present or may present a risk to the environment or to human health.

Based on the information obtained through the categorization process, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or presented an intermediate potential for exposure (IPE) and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

The Ministers therefore published a notice of intent in the *Canada Gazette*, Part I, on December 9, 2006 (Canada 2006), which challenged industry and other interested stakeholders to submit, within specified timelines, specific information that may be used to inform risk assessment and to develop and benchmark best practices for the risk management and product stewardship of those substances identified as high priorities.

The substance phenol, (1,1-dimethylethyl)-4-methoxy- was identified as a high priority for assessment of human health risk because it was considered to present IPE and had been classified by other agencies on the basis of carcinogenicity. The Challenge for this substance was published in the *Canada Gazette* on January 31, 2009 (Canada 2009). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of technical information were received.

Although phenol, (1,1-dimethylethyl)-4-methoxy- was determined to be a high priority for assessment with respect to human health, it was not considered to meet the criteria for persistence, bioaccumulation potential or inherent toxicity to aquatic organisms. Therefore, this assessment focuses principally upon information relevant to the evaluation of risks to human health.

Screening assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of CEPA 1999. Screening assessments examine

scientific information and develop conclusions by incorporating a weight of evidence approach and precaution[1].

This final screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents and stakeholder research reports, as well as from recent literature searches, up to July 2009 for the sections of the document relevant to human health and up to October 2009 for the ecological sections of the document. Key studies were critically evaluated; modelling results may have been used to reach conclusions. Evaluation of risk to human health involves consideration of data relevant to estimation of exposure (non-occupational) of the general population, as well as information on health hazards (based principally upon the weight of evidence assessments of other agencies that were used for prioritization of the substance). Decisions for human health are based upon the nature of the critical effect and/or margins between conservative effect levels and estimates of exposure, taking into account confidence in the completeness of the identified databases on both exposure and effects, within a screening context. The screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents a summary of the critical information upon which the conclusion is based.

This final screening assessment was prepared by staff of the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological and human health portions of this assessment have undergone external written peer review/consultation. Comments on the technical portions relevant to human health were received from scientific experts selected and directed by Toxicology Excellence for Risk Assessment (TERA), including Dr. John Christopher (California Office of Environmental Health Assessment), Dr. Michael Jaycock (The Lifeline Group) and Ms. Joan Strawson (TERA). Additionally, the draft of this screening assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment Canada. Approaches used in the screening assessments under the Challenge have been reviewed by an independent Challenge Advisory Panel.

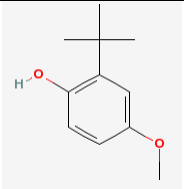
The critical information and considerations upon which the final screening assessment is based are summarized below.

[1] A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and the use of consumer products. A conclusion under CEPA 1999 on the substances in the Chemicals Management Plan (CMP) Challenge Batches 1-12 is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the Controlled Products Regulations, which is part of regulatory framework for the Workplace Hazardous Materials Information System [WHMIS] for products intended for workplace us

Substance Identity

For the purposes of this document, this substance will be referred to as butylated hydroxyanisole (BHA). Information on the identity of BHA is summarized in Table 1.

Table 1. Substance identity

CAS RN	25013-16-5
DSL name	Phenol, (1,1-dimethylethyl)-4-methoxy-
NCI names[1]	*t*-Butyl-*p*-hydroxyanisole (ENCS) *tert*-Butyl-4-methoxyphenol (EINECS) 1,1-(Dimethylethyl)-4-methoxyphenol (ECL) Phenol, (1,1-dimethylethyl)-4-methoxy- (AICS, ASIA-PAC, DSL, NZIoC, PICCS, SWISS, TSCA)
Other names	Antioxyne B BHA BHA (antioxidant) BOA BOA (antioxidant) 2(3)-*tert*-Butyl-4-hydroxyanisole *tert*-Butyl-4-hydroxyanisole Butylated hydroxyanisole Butylhydroxyanisole *tert*-Butylhydroxyanisole Phenol, *tert*-butyl-4-methoxy-
Chemical group (DSL stream)	Discrete organics
Major chemical class or use	Phenols
Major chemical subclass	Hydroxyanisoles; *tert*-butyls
Chemical formula	$C_{11}H_{16}O_2$
Chemical structure	
SMILES[2]	COc1ccc(O)c(c1)C(C)(C)C
Molecular mass	180.25 g/mol

[1] National Chemical Inventories (NCI). 2007: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); DSL, Domestic Substances List; ECL (Korean Existing Chemicals List); EINECS (European Inventory of Existing Commercial Chemical Substances); ENCS (Japanese Existing and New Chemical Substances); National Chemical Inventories; NZIoC, New Zealand Inventory of Chemicals; PICCS (Philippine Inventory of Chemicals and Chemical Substances); SWISS, Swiss Giftliste 1 and Inventory of Notified New Substances; and TSCA (Toxic Substances Control Act Chemical Substance Inventory).

[2] Simplified Molecular Input Line Entry System

Physical and Chemical Properties

Table 2 contains experimental and predicted physical and chemical properties of BHA that are relevant to its environmental fate. The table also contains experimental physical and chemical properties for a structural analogue, 4-*tert*-butylphenol. A comparison between the chemical structures of BHA and 4-*tert*-butylphenol can be found in Table 3.

Table 2. Physical and chemical properties of BHA and an analogue, 4-*tert*-butylphenol

Data in Table 2 are for BHA unless otherwise noted.

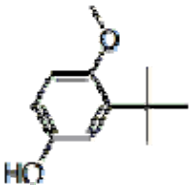
Property	Type	Value[1]	Temperature (°C)	Reference
Melting point (ºC)	Experimental	51		PhysProp 2006
	Modelled	66.21		MPBPWIN 2000
Boiling point (ºC)	Experimental	268		PhysProp 2006
	Modelled	265.53		MPBPWIN 2000
Density (kg/m^3)	Not available			
Vapour pressure (Pa)	Modelled	0.312		MPBPWIN 2000
Henry's Law constant (Pa·m^3/mol)	Modelled	8.675×10^{-3} Bond method (8.562×10^{-8} atm·m^3/mol) 0.119 Group method (1.171×10^{-6} atm·m^3/mol)	25	HENRYWIN 2000
Log K_{ow} (dimensionless)	Modelled	3.5	25	KOWWIN 2000
	Experimental (4-*tert*-butylphenol)	3.29		OECD 2000
Log K_{oc} (dimensionless)	Modelled	3.143		PCKOCWIN 2000
Water solubility (mg/L)	Modelled	212.8	25	WSKOWWIN 2000

Property	Type	Value[1]	Temperature (°C)	Reference
	Experimental (4-*tert*-butylphenol)	610		OECD 2000
pK_a	Modelled	11.83		ACD/pK_aDB 2005

Abbreviations: K_{oa}, octanol–air partition coefficient; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; pK_a, acid dissociation constant.

1 Values and units in parentheses represent those originally estimated by the models.

Table 3. Comparison of BHA and a structural analogue (4-*tert*-butylphenol)

CAS RN	Common name	Molecular weight (g/mol)	DSL name	Chemical structure	Experimental data available
25013-16-5	BHA	180.25	Phenol, (1,1-dimethylethyl)-4-methoxy-		Melting point, boiling point, aquatic toxicity, bioaccumulation
98-54-4	4-TBP	150	4-*tert*-butylphenol		log K_{ow}, water solubility, biodegradation, bioaccumulation

Models based on quantitative structure–activity relationships (QSARs) were used to generate data for some of the physical and chemical properties of BHA. Many of these models are based on fragment addition methods (i.e., they rely on the structure of a chemical). Since these models accept only the neutral form of a chemical as input (in SMILES form), the modelled values shown in Table 2 are for the neutral form of BHA. In addition, the high pK_a of 11.83 indicates that the compound is not expected to be ionized in an environmentally relevant pH range (6–9). As relatively simple organic chemicals like BHA are well represented in the model training sets, there is a reasonably high level of confidence in the model predictions.

4-*tert*-Butylphenol was chosen as an analogue to provide additional read-across experimental data on the basis of its strong structural similarity to BHA and because it is recognized to be in the same chemical class of tertiary butylphenols (Remberger et al. 2003). There are some structural differences between the two substances. BHA contains an extra oxygen atom and methyl group attached to the phenol ring, and the hydroxy group is situated at a different location on the ring.

Sources

In 2006, no BHA was manufactured in Canada at quantities equal to or greater than the reporting threshold of 100 kg. However, between 100 and 1000 kg of BHA was imported into Canada, while between 1000 and 10 000 kg of BHA was used in Canada, alone, in a product, in a mixture or in a manufactured item (Environment Canada 2009a).

The US Environmental Protection Agency lists BHA as a "high production volume" chemical (NCBI 2008). In 1998, the US Geological Survey reported that 11% of 126 samples of wastewater from diverse sources contained BHA (Brown et al. undated).

Previously received information from the Domestic Substances List nomination (1984–1986) showed that the total quantity of BHA reported as imported into, manufactured in or in commerce in Canada during the calendar year 1986 was in the range from 100 000 to 1 000 000 kilograms (Environment Canada 1988).

Uses

BHA is an antioxidant that can be used in a large number of foods according to Division 16, Table XI, of the *Food and Drug Regulations*. BHA may be added to certain foods up to the maximum concentrations specified in the *Food and Drug Regulations* or can be used in combination with butylated hydroxytoluene, propyl gallate and/or *tert*-butyl hydroquinone (TBHQ), such that the total maximum level of use of all antioxidants is the same as the maximum use level for BHA alone (Canada [1978]). In the United States, BHA is a generally recognized as safe (GRAS) compound when its content is not greater than 0.02% by weight of the total fat or oil content of the food (NTP 2005).

In fats and fat-containing foods, the antioxidant property of BHA delays the deterioration of flavours and odours and substantially increases the shelf life (NTP 2005). BHA is appropriate for use in foods baked or fried in animal oils because of its high thermal stability and ability to remain active in foods cooked in this manner (NTP 2005). BHA was reported to be used in Canada in 2006 as an antioxidant in food (Environment Canada 2009a) and may have applications in a wide range of foods, such as fats and oils, salad dressings, ready-to-eat cereals, baked foods, dehydrated potato products, food and beverage mixes, snack foods, confectionery, chewing gum and ready-to-eat sauces and puddings.

In Canada, BHA is reported to be used as a stabilizer in a few plastics intended for direct food contact; however, the contribution to overall intake from this source is negligible (2009 email from Risk Management Bureau, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced).

BHA is also an antioxidant and preservative in animal feed, cosmetics, rubber and petroleum products. It is a primary antioxidant used in animal feeds, as it retards the oxidation of vitamins A and E, carotene, xanthophyll pigments, rendered fats and vegetable oils. It is an effective stabilizer for essential oils, paraffin and polyethylenes (NTP 2005).

BHA is used as a preservative and antioxidant in pharmaceutical preparations (NTP 2005). It is listed in the Natural Health Products Ingredient Database and the Licensed Natural Health Products Database as a non-medicinal ingredient for use as an antioxidant and antimicrobial preservative in final pharmaceutical products, natural health products and veterinary products manufactured in Canada (2009 email from Risk Management Bureau, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced). In natural health products, BHA has an acceptable daily intake (ADI) of 0.5 mg/kg body weight (kg-bw) (NHPID 2009).

BHA is a constituent of personal care products, such as deodorants, shampoos and body lotions, in Canada (2009 email from Risk Management Bureau, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced). In the United States, BHA is listed among the most frequently used cosmetic ingredients (US FDA 2009).

In Canada, BHA is a List 2 formulant in pesticide products, at concentrations ranging from 0.000 04% to 1% (2009 email from Risk Management Bureau, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced). It is a stabilizer or fragrance in rodenticides, insecticides (home garden), fungicides, bactericides (pulp and paper), insecticidal shampoos (cats and dogs), insect repellents (lotion, towelettes) and antibacterial glass and surface cleaners. BHA is also listed as an inert ingredient in pesticide products in the United States (US EPA 2004).

BHA is on the Ingredient Disclosure List (concentration 1% by weight) of the *Hazardous Products Act* (Canada 1988).

Releases to the Environment

BHA is not included in Canada's National Pollutant Release Inventory (NPRI 2009). Similarly, BHA was not identified in a search of the US Toxics Release Inventory in 2006, the most recent reporting year (TRI 2006).

Environmental Fate

Based on its physical and chemical properties (Table 2), the results of Level III fugacity modelling (Table 4) suggest that BHA will reside predominantly in soil and water, depending on the compartment of release.

Table 4. Results of Level III fugacity modelling for BHA (EQC 2003)

Substance released to:	Percentage of substance partitioning into each compartment			
	Air	Water	Soil	Sediment
Air (100%)	24.6	10.8	63.8	0.78
Water (100%)	0.02	93.2	0.06	6.72
Soil (100%)	0.00	0.58	99.4	0.04

The relatively high acid dissociation constant (pK_a) of 11.83 for the acidic functional group indicates that half of the chemical will be dissociated at pH 11.83. In water bodies at environmentally relevant pH (6–9), 100% will be undissociated, which indicates that biotic exposure to BHA will be to the neutral chemical. The relatively low proportion of dissociated chemical also indicates that it is appropriate to predict partitioning behaviour using the log K_{ow} and log K_{oc}.

Persistence and Bioaccumulation Potential

Environmental Persistence

No experimental degradation data for BHA have been identified. However, an experimental biodegradation test is available for the analogue 4-*tert*-butylphenol.

Table 5a presents empirical biodegradation data that show 98% biodegradation over 28 days in a ready biodegradation test for 4-*tert*-butylphenol (OECD 2000). This test indicates that the half-life in water is likely to be much shorter than 182 days (6 months) and that the substance is therefore not likely to persist in that environmental compartment.

Table 5a. Empirical data for degradation of 4-*tert*-butylphenol

Medium	Fate process	Degradation value	Degradation endpoint (units)	Reference
Water	Biodegradation	98	Biodegradation (%)	OECD 2000

Given the ecological importance of the water compartment, the fact that most of the available models apply to water and the fact that BHA is expected to be released to this compartment, persistence primarily in water was examined using predictive QSAR models for biodegradation. Table 5b summarizes the results of available QSAR models for degradation in various environmental media.

Table 5b. Modelled data for degradation of BHA

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Air			
Atmospheric oxidation	AOPWIN 2000	$t_{½}$ = 0.297 day	<2
Ozone reaction	AOPWIN 2000	n/a[1]	n/a
Water			
Hydrolysis	HYDROWIN 2000	n/a[1]	n/a
Biodegradation (aerobic)	BIOWIN 2000 Submodel 3: Expert Survey	2.58[2] "biodegrades slowly"	>182[3]

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
	(ultimate biodegradation)		
Biodegradation (aerobic)	BIOWIN 2000 Submodel 4: Expert Survey (primary biodegradation)	3.55[2] "biodegrades fast"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 5: MITI linear probability	0.53[3] "biodegrades fast"	<182
Biodegradation (aerobic)	BIOWIN 2000 Submodel 6: MITI non-linear probability	0.49[3] "biodegrades fast"	<182
Biodegradation (aerobic)	TOPKAT 2004 Probability	0.293 "may biodegrade slowly"	≥182 uncertain
Biodegradation (aerobic)	CATABOL ©2004–2008 % BOD	% BOD = 27.3 "may biodegrade slowly"	≥182 uncertain

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade & Industry, Japan; n/a, not applicable; $t_{½}$, half-life.

[1] Model does not provide an estimate for this type of structure.

[2] Output is a numerical score from 0 to 5.

[3] Output is a probability score.

In air, a predicted atmospheric oxidation half-life of about 0.3 day (Table 5b) demonstrates that this substance is likely to be rapidly oxidized. The substance is not expected to react appreciably with other photo-oxidative species in the atmosphere, such as ozone, nor is it likely to degrade via direct photolysis. Therefore, it is expected that reactions with hydroxyl radicals will be the most important fate process in the atmosphere for BHA. With a half-life of about 0.3 day via reactions with hydroxyl radicals, BHA is considered to be not persistent in air.

In water, a hydrolysis half-life could not be predicted and is not relevant, as there are no hydrolyzable groups (Table 5b).

There are conflicting model results for biodegradation. The result of BIOWIN (2000) Submodel 4 (primary survey model) suggests that the substance biodegrades rapidly and has a primary half-life of <182 days. The identity of the degradation products are, however, unknown. The results of BIOWIN (2000) Submodels 5 and 6 suggest that this compound will undergo a relatively fast ultimate biodegradation, while Submodel 3 suggests that this compound will biodegrade slowly. The result of the model CATABOL (©2004–2008) also suggests that BHA may undergo a slower rate of ultimate degradation and that the half-life may be greater than or equal to 182 days. However, the predicted BOD of 27% from CATABOL (©2004–2008) is not less than 20%, which is associated with very persistent compounds. TOPKAT (2004) predicts that BHA will biodegrade relatively slowly, but there is not a large margin (0.07) between this prediction and the prediction that it may biodegrade quickly. Therefore, the models indicate that the substance likely undergoes significant primary biodegradation over 182 days, and a majority of the models suggest that it undergoes significant ultimate degradation in 182 days. The experimental biodegradation data for the structural analogue 4-*tert*-butylphenol

(Table 5a) also demonstrate ready biodegradation. Therefore, considering all model results, experimental biodegradation results for an analogue and structural features, most of the reliable evidence suggests that the biodegradation half-life of BHA is <182 days in water.

Using an extrapolation ratio of 1:1:4 for water:soil:sediment biodegradation half-lives (Boethling et al. 1995), the half-life in soil is also <182 days. However, since CATABOL (©2004–2008) and TOPKAT (2004), which are both based on MITI data, predict somewhat slower biodegradation, and since the ultimate degradation half-life in water is likely 90–182 days, the half-life in sediments is likely ≥365 days. This indicates that BHA is not expected to be persistent in soil but is expected to be persistent in sediment.

Based on the experimental analogue and predicted data (Tables 5a and 5b), BHA does not meet the persistence criteria for air, water or soil (half-life in air ≥2 days; half-lives in soil and water ≥182 days), but it does meet the persistence criterion for sediment (half-life in sediment ≥365 days), as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

An experimental study on the retention of BHA in Atlantic salmon (*Salmo salar*) was commissioned by the Norwegian National Institute of Nutrition and Seafood Research (NIFES 2007). Salmon were exposed to BHA in food for 84 days, followed by a 14-day depuration period. The results of the study showed that levels of BHA in salmon fillets were dose dependent throughout the exposure period, with a highest concentration of 0.183 mg/kg at the highest exposure level of 225 mg/kg food. However, the concentrations of BHA did not significantly increase during the study, indicating that accumulation is prevented by rapid elimination/catabolism. In addition, the concentration of BHA in salmon fillets was below the limit of detection (7 µg/kg) after the 2-week depuration period. As this study was not set up in a standard manner, it is given a lower weight of importance in the lines of evidence. However, the experimental results do provide information that suggests a low potential for bioaccumulation, as BHA seemed to be rapidly eliminated following uptake.

Table 6a presents an empirical bioconcentration factor (BCF) value for the structural analogue 4-*tert*-butylphenol in fish.

Table 6a. Empirical data for bioaccumulation of 4-*tert*-butylphenol

Test organism	Endpoint	Value wet weight (L/kg)	Reference
Fish	BCF	120	OECD 2000

Since no standard experimental bioaccumulation factor (BAF) or BCF data for BHA were available, a predictive approach was applied using available BAF and BCF models, as shown in Table 6b. According to the *Persistence and Bioaccumulation Regulations* (Canada 2000), a substance is bioaccumulative if its BCF or BAF is ≥5000; however,

measures of BAF are the preferred metric for assessing the bioaccumulation potential of substances. This is because BCF may not adequately account for the bioaccumulation potential of substances via the diet, which predominates for substances with log K_{ow} > ~4.0 (Arnot and Gobas 2003). Kinetic mass balance modelling is in principle considered to provide the most reliable prediction method for determining the bioaccumulation potential because it allows for metabolism correction as long as the log K_{ow} of the substance is within the log K_{ow} domain of the model.

Table 6b. Modelled data for bioaccumulation

Test organism	Endpoint	Value (L/kg wet weight)	Reference
Fish	BAF	110.5	Arnot and Gobas 2003 (BCF middle trophic level)
Fish	BCF	110.6	Arnot and Gobas 2003 (BCF middle trophic level)
Fish	BCF	705	OASIS Forecast 2005
Fish	BCF	57	BCFBAF 2008

The Arnot and Gobas middle trophic level BCF and BAF predictions include biotransformation rate estimates (k_M = 1.8 d^{-1} for 10 g fish). This is consistent with Atlantic salmon bioaccumulation study (NIFES 2007) which suggested rapid elimination and with the empirical BCF for the structural analogue 4-*tert*-butylphenol in fish.

The available evidence indicates that BHA is expected to have low bioaccumulation potential due to its physical and chemical properties (i.e., relatively low modelled and analogue K_{ow}, moderate modelled and analogue water solubility), its rapid elimination and its low read-across and modelled BAF and BCF values. Therefore, BHA does not meet the bioaccumulation criteria (BCF, BAF ≥5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

Ecological Effects Assessment

A summary of experimental ecological effects data is presented in Table 7.

Table 7. Empirical data for aquatic toxicity

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Chlorella vulgaris (alga)	Chronic (48 h)	EC_{50}	7.6 (42 μM)	Jos et al. 2005[1]
Allium cepa (plant)	Chronic (growth inhibition, 72 h)	EC_{50}	35 (194 μM)	Jos et al. 2005[1]
Vibrio fischeri (bacterium)	Acute (5, 15, 60 min)	EC_{50}	0.21 (1.17 μM)	Jos et al. 2005

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Daphnia magna	Acute (48 h)	EC_{50}	3.6 (20 µM)	Jos et al. 2005
Zebra mussel (*Dreissena polymorpha*)	Acute (48 h)	LC_{50}	34.2 (mean of two samples: 3.4 and 65)	Cope et al. 1997
Rainbow trout (*Oncorhynchus mykiss*)	Acute (48 h)	LC_{50}	1	Cope et al. 1997
Channel catfish (*Ictalurus punctatus*)	Acute (48 h)	LC_{50}	1.5	Cope et al. 1997
Bluegill (*Lepomis macrochirus*)	Acute (48 h)	LC_{50}	4.8	Cope et al. 1997
Medaka, high eyes (*Oryzias latipes*)	Acute (48 h)	LC_{50}	4.3 (mean of three values ranging from 2.5 to 5.3)	Tsuji et al 1986

Abbreviations: EC_{50} (median effective concentration), the concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms; LC_{50} (median lethal concentration), the concentration of a substance that is estimated to be lethal to 50% of the test organisms.

The alga *Chlorella vulgaris* was the most sensitive organism in the chronic data set, with a reported 48-hour cell growth inhibition EC_{50} of 7.6 mg/L.

Vibrio fischeri was the most sensitive species in the acute data set, with a mean EC_{50} value for inhibition of bioluminescence of 0.21 mg/L after 5, 15 and 60 minutes of exposure. The toxicity of BHA to *V. fischeri* has been hypothesized to be due to an uncoupling of oxidative phosphorylation and inhibition of respiration by a direct interaction with the electron transport chain (Cope et al. 1997). Rainbow trout were the second most sensitive species in the acute data set, with a mean 48-hour LC_{50} value of 1 mg/L (Cope et al. 1997). Fish LC_{50} data were quite consistent, ranging only slightly from 1 to 4.8 mg/L.

The range of experimental aquatic toxicity data considered indicate that the substance is moderately to highly hazardous to aquatic organisms, with acute LC_{50}/EC_{50} values ranging from 0.21 mg/L to 35 mg/L (Table 7).

In addition to having moderate to high toxicity, BHA has been described as an endocrine disrupting compound in the scientific literature (Goo Kang et al. 2005**;** Jobling et al. 1995; Jos et al. 2005; PAN 2009 and Scippo 2004). However, there is conflicting evidence about the degree of endocrine disruption and whether the chemical is estrogenic or anti-estrogenic. Therefore, the interpretation of this information in an environmental context is unclear.

Due to the high degree of uncertainty associated with the ecological relevance of the current endocrine disruption research on BHA and the conflicting results related to estrogenic effects, the assessment uses conventional toxicological endpoints of concern (i.e., impaired growth and mortality; Table 7) together with assessment factors to protect against possible subtle effects of long-term exposure to characterize the ecological risk.

Ecological Exposure Assessment

BHA was found in samples from streams across the United States at a maximum concentration of 2×10^{-4} mg/L (Kolpin et al. 2002). One of three samples of surface river water collected in Illinois and Minnesota in 1998 contained BHA (0.3 µg/L) (Brown et al. undated). In the same study, all eight samples of effluent from sewage treatment plants (STPs) (in Illinois, Minnesota, Michigan and Wisconsin) contained BHA (0.2–1.3 µg/L). A study by Soliman et al. (2004) found BHA in all samples from treated effluents at three water treatment plant sites in southern California; the concentrations of BHA found in these samples ranged from 0.02 to 0.13 µg/L. BHA was detected in the effluent from three STPs in Sweden at concentrations of 0.5, 0.5 and 1 µg/L (Paxéus 1996).

Rudel et al. (1998) analyzed septage and wastewater in Cape Cod, Massachusetts. BHA was detected in five of five samples of untreated septage (range 0.13–0.53 µg/L), in two of four samples of untreated wastewater (0.025 and 0.05 µg/L) and in none of three samples of treated septage/wastewater. Samples were also taken from groundwater monitoring wells in or near three municipal landfills. BHA was not detected (detection limit 0.0066 µg/L) in four samples from the groundwater influenced by landfill leachate, and one of five samples from a landfill/septage lagoon contained BHA below the quantification limit (0.002 µg/L).

Both raw sewage and final treated effluent were sampled at six treatment plants in three states within the western continental United States in 2005 (Drewes and Snyder 2009). The mean concentrations in six samples of each of raw influent and final effluent were 160 ng/L and 70 ng/L, respectively, as analyzed by liquid chromatography with tandem mass spectrometry. Analysis by gas chromatography with tandem mass spectrometry yielded means of 190 ng/L and 90 ng/L for raw influent and final effluent, respectively.

These exposure studies emphasize the importance of water as a receiving medium of BHA in the environment. The highest recorded concentrations found in the literature were from samples taken in 1998 by Brown et al. (undated) in United States rivers and wastewaters and ranged from 0.2×10^{-3} to 1.3×10^{-3} mg/L, with the highest value being for wastewater from a municipal sewage treatment plant.

Characterization of Ecological Risk

The approach taken in this ecological screening assessment was to examine various supporting information and develop conclusions based on a weight of evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from conservative risk quotient calculations as well as information on persistence, bioaccumulation, toxicity, sources and fate of the substance.

As described previously, BHA meets the persistence criteria for sediment as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000). It is not persistent in water, soil or air and is expected to have a low bioaccumulation potential.

Consumer Releases

As BHA can be found in consumer products and is reported to be released to water, a specific scenario was developed to calculate a risk quotient for aquatic organisms. A conservative predicted environmental concentration (PEC) is based on the highest known municipal effluent concentration from the Brown et al. (undated) study, 1.3×10^{-3} mg/L.

A conservative predicted no-effect concentration (PNEC) was derived from the second lowest toxicity value identified, an acute value for rainbow trout of 1 mg/L (Cope et al. 1997). The rainbow trout endpoint value was chosen over the bacterial endpoint as it is considered more representative of other aquatic endpoints and is still protective. The 1 mg/L acute toxicity value for rainbow trout was divided by an assessment factor of 100 to account for uncertainties related to extrapolation from an acute to a chronic value and extrapolation from a laboratory LC_{50} to a no-effect value in the field. This yielded a PNEC of 0.01 mg/L.

The resulting conservative risk quotient (PEC/PNEC: 0.0013/0.01) of 0.13 for sensitive pelagic species is below 1. This indicates that exposure values are unlikely to be high enough to cause harm to aquatic organisms.

In addition Mega Flush, Environment Canada's spreadsheet model for estimating down-the-drain releases from consumer uses, was employed to estimate the potential concentration of BHA in multiple water bodies receiving STP effluents to which consumer products containing the substance may have been released (Environment Canada 2009b). The spreadsheet model is designed to provide these estimates based on conservative assumptions regarding the amount of the substance used and released by consumers.

By default, losses from use are assumed to be 100%, consumer use of the substance is assumed to be over 365 days/year and the flow rate of receiving waters at all sites is assumed to be low (10th percentile) values. These estimates are made for approximately 1000 release sites across Canada, which account for most of the major STPs in the country.

The equation and inputs used to calculate the PEC of BHA in the receiving water bodies are described in Environment Canada (2009c). A scenario was run with the total volume reported to be used in Canada for the year 2006. Additionally, in this scenario some default parameter values were modified based on the information available, increasing its realism. In particular, a primary STP removal rate of 12% was obtained from the SimpleTreat STP model (SimpleTreat 1997) and a combined primary and secondary STP removal rate of 72% was obtained from the ASTreat STP model (ASTreat 2006) The

overall effect of the parameter values chosen is to make this scenario moderately conservative.

Using this scenario, the tool estimates that the PEC in the receiving water bodies ranges from 3.2×10^{-6} to 4.3×10^{-3} mg/L. The resulting range of risk quotients (PEC/PNEC) is 3.2×10^{-4} to 0.43 (3.2×10^{-6}/0.01 to 4.3×10^{-3}/0.01) for sensitive pelagic species – all values being below 1. Given the conservative nature of this scenario – particularly the assumption that 100% of the substance used by consumers is lost down-the-drain - this indicates that exposure values are unlikely to be high enough to cause harm to aquatic organisms.

Industrial Releases

Since BHA is used industrially, and as a significant proportion of BHA may be released to water in Canada, site-specific scenarios were used to estimate the concentration of BHA resulting from industrial discharges at five sites known to use this substance. The scenario at each site was made conservative by assuming that the total quantity used is the maximum possible for each notifier based on the quantities reported for 2006, and that the loss to sewers is high, at 5% of the total quantity, resulting from the cleaning of chemical containers and process equipment. The five separate scenarios also assume that the releases occur 250 days/year, typical for small and medium-sized facilities, and are sent to a local STP with site-specific removal rates that range from 12% (primary treatment) to 72% (primary and secondary treatment). Based on the above assumptions, and applying site-specific dilution factors (to a maximum factor of 10) to the resulting effluent concentrations, the five industrial release scenarios result in a range of aquatic concentrations from to 5.3×10^{-5} to 2.8×10^{-3} mg/L.

The resulting range of conservative risk quotients (PEC/PNEC) of 5.3×10^{-3} (5.3×10^{-5} /0.01) to 0.28 (2.8×10^{-3} /0.01) for sensitive pelagic species is below 1, which indicates that exposure values are unlikely to be high enough to cause harm to aquatic organisms.

Since the majority of releases of BHA are likely to water through consumer uses or at industrial sites and as the results of fugacity modelling indicate that most of the substance discharged to water will remain in that compartment, significant exposure of organisms at other locations or in media other than water is unlikely.

The available information suggests that BHA does not have the potential to cause ecological harm in Canada.

Uncertainties in Evaluation of Ecological Risk

There are uncertainties associated with the use of QSAR models to estimate persistence and bioaccumulation, but since BHA is a relatively simple organic molecule, there is a reasonably high level of confidence in the model predictions. In addition, QSAR models were based on the estimated values for some of the key physical and chemical properties,

including vapour pressure, water solubility and K_{ow}. There is also uncertainty related to the reported endocrine disruption potential of BHA and its ecological significance, which were addressed in part by using assessment factors to extrapolate from conventional measures of acute effects to a chronic no effect value in the field which is intended to protect against more subtle effects of long-term exposure.

Potential to Cause Harm to Human Health

Exposure Assessment

Environmental Media and Food

Concentrations of BHA were not identified in drinking water in Canada (2009 email from Water, Air and Climate Change Bureau, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced).

In the United States, BHA is a substance that is "known or anticipated to occur in public water systems" (US EPA 2008). BHA was "tentatively identified" in raw and finished samples in a survey of public water supplies in New Jersey (NJDEP 2003). Stiles et al. (2008) collected both raw and finished water at treatment facilities in northern New Jersey; BHA was detected, but not quantified, in both raw and treated water. During 1999–2000, the US Geological Survey collected water samples from 139 streams across 30 states at sites that were downstream of intense urbanization or livestock production; BHA was identified in 2 of 85 samples (maximum concentration 0.2 µg/L) (Kolpin et al. 2002). BHA was detected in surface water from the Colorado River (24 ng/L), but not in samples from the Sacramento–San Joaquin River delta (Soliman et al. 2007).

BHA has been detected in surface water of streams and rivers in Germany (maximum concentration 35 ng/L) (Boltz et al. 2001). BHA was consistently detected (concentration not reported) in samples of the river Po (Italy) throughout 1994–1996 (Davi and Gnudi 1999). BHA was detected in 6 of 20 samples of effluent from municipal sewage treatment plants and 3 of 30 river water samples in Spain (Gomez et al. 2009).

Estimates of intake of BHA from drinking water were calculated based upon the maximum concentration of 0.2 µg/L identified in the 1999–2000 survey by the US Geological Survey (Kolpin et al. 2002). The maximum estimated intake (for formula-fed infants) was 0.02 µg/kg-bw per day, which was negligible in comparison with estimated intakes from food and personal care products (see below).

BHA is an antioxidant that can be used in a large number of foods, according to Division 16, Table XI, of the *Food and Drug Regulations*. BHA may be added to certain foods up to the maximum concentrations specified in the *Food and Drug Regulations* or can be used in combination with butylated hydroxytoluene, propyl gallate and/or TBHQ, such that the total maximum level of use of all antioxidants is the same as the maximum use

level for BHA alone. The maximum use levels are based on either a percentage of the total lipid content of a given food or the total weight of the food (Canada [1978]). Actual use levels of BHA were available for a few foods that were considered in the exposure assessment. For other foods, it was assumed that these always contained BHA at the maximum level of use, except where BHA can be used in combination with other antioxidants. In such cases, the BHA concentration in food was assumed to be a fraction (one-half, one-third or one-quarter, depending on the number of antioxidants permitted) of the maximum permitted use level of the antioxidant mixture. Certain foods may also contain BHA as a result of the addition of vitamin A or D, each of which is also permitted to contain BHA.

Of the foods that are permitted to contain BHA, those that make a significant contribution to the diet or are popular foods for children were included in the exposure assessment, while foods that make an insignificant contribution to exposure were excluded. In addition, all foods (except cow's milk) to which vitamins A and D must be added were excluded from the assessment, as the contribution of BHA to foods from its use in vitamin A and D preparations is very low. BHA exposure from the use of vitamins A and D in cow's milk was included, because milk is commonly consumed by children. However, exposure to BHA from milk was also determined to be negligible. Approximately 40 food categories, such as salad dressing, which includes foods such as mayonnaise-based and oil and vinegar–based dressings, were included in the exposure assessment.

Food intake data were obtained from the Canadian Community Health Survey (CCHS), Cycle 2.2 on Nutrition (Statistics Canada 2004). One-day, all-person intakes reported by survey respondents were employed, and no adjustments were made to account for usual daily intakes, a statistical adjustment that approximates the distribution of long-term average daily exposure. Exposure figures were adjusted for body weight (both self-reported and measured) such that they would be representative of the Canadian population. Body weights are not reported by the CCHS for children less than 2 years old; hence, values derived from the Continuing Survey of Food Intakes by Individuals (CSFII 1994–96, 1998) were used.

A probabilistic exposure assessment was conducted. The estimated mean all-person daily intakes of BHA from the diet for all age classes of consumers are reported in Appendix 1. Mean intakes ranged from 0.023 to 0.095 mg/kg-bw per day, depending on age class.

Several foods drive exposure to BHA in infants less than 1 year of age; foods that account for more than 10% of the total exposure to BHA in this age category include powdered dessert mixes, ready-to-eat cereals, crackers and bread. Fats and oils drive exposure for all age groups of consumers other than infants less than 1 year of age, accounting for 16–24% of total BHA exposure in consumers more than 1 year of age. Other foods that each account for more than 10% of the total exposure to BHA for people more than 1 year of age are margarine, salad dressings, dry desserts, bread, ready-to-eat cereals and powdered beverage mixes.

Although some food categories that would contribute minor amounts to BHA exposure were not included in the assessment, dietary exposure to BHA may have been overestimated, as insufficient quantitative information was available to account for the following factors: actual use levels of BHA in relation to the maximum permitted levels of use specified in the *Food and Drug Regulations*; actual use levels of BHA in combination with other antioxidants; possible discontinued use of BHA by manufacturers in some foods in which it is permitted; and possible losses of BHA in foods during processing, transport and storage.

Personal Care Products

BHA is a constituent of personal care products, such as deodorants, shampoos and body lotions (2009 email from Risk Management Bureau, Health Canada, to Risk Assessment Bureau, Health Canada; unreferenced). No studies were identified that reported the *in vivo* absorption of BHA in experimental animals or humans. Schumann (1991) reported the *in vitro* absorption of BHA through human skin. In three parallel trials with abdominal skin from a 24-year-old woman, BHA was applied in water-in-oil ointment for 16 hours. The highest concentration in the chamber fluid was 5.34% of the applied dose, after whole skin stripping. Therefore, 5% absorption was applied in the ConsExpo (2006) modelling for personal care products. The absence of toxicity noted in dermal exposure studies (see Health Effects Assessment below) is consistent with the estimated low dermal absorption value.

Estimates of intake from personal care products are presented in Appendix 2. Estimates varied, depending upon the concentration of BHA in the product, the body surface area to which the product was applied and the duration of exposure. The available data for BHA concentration were reported in ranges. The lowest range was from 0% to 0.1%. The range of 0.3–1% was selected as the range for calculating maximum (conservative) intake, as almost all of the several thousand BHA-containing personal care products available in Canada contain up to a maximum concentration of 1% BHA, with the majority containing at most a maximum of 0.1% BHA. Although a few products were reported to contain concentrations higher than 1%, they were considered to be not representative of the database as a whole. Products that were applied more frequently and that covered larger surfaces of the body (shampoos, body lotion) contributed most to the combined intake from frequently used products. For these products, the 95th-percentile concentration of BHA was used to estimate intake, to more conservatively reflect exposure.

The ranges of combined intakes of BHA from frequently used personal care products are presented in Table 8. The maximum estimated intake of BHA is 0.07 mg/kg-bw per day, for children aged 6 months to <5 years, followed closely by children 5–12 years of age (0.06 mg/kg-bw per day) and women (0.06 mg/kg-bw per day).

Table 8. Intakes of BHA from dermal exposure to frequently used personal care products

Product	Intake (mg/kg-bw per day)[1]	
	Average	**Maximum**
Infants		
Baby cream	0.004	0.04
Total	0.004	0.04
Children 6 months to <5 years		
Shampoo[2]	0.0023	0.009 89 (using 0.43% BHA, 95th percentile)
Body lotion, applied twice daily	0.0177	0.0583 (using 0.33% BHA, 95th percentile)
Total	0.02	0.07
Children 5–12 years		
Shampoo	0.0015	0.006 46 (using 0.43% BHA, 95th percentile)
Body lotion, applied twice daily	0.0148	0.0489 (using 0.33% BHA, 95th percentile)
Total	0.02	0.06
Men		
Shaving cream	0.000141	0.000141 (all shaving preparations are in 0–0.1% range)
Aftershave	0.000 846	0.000 846 (all shaving preparations are in 0–0.1% range)
Hair gel	0.000 207	0.002 07
Deodorant spray	0.003 66	0.003 66 (all deodorants are in 0–0.1% range)
Shampoo	0.001	0.004 32 (using 0.43% BHA, 95th percentile)
Total	0.006	0.01
Women[3]		
Deodorant spray	0.003 66	0.00 366 (all deodorants are in 0–0.1% range)
Facial makeup	0.0005 64	0.005 64
Eau de toilette	0.001 28	28 (no fragrances in this range; used value for 0–0.1% range)
Hairspray	0.000 507	0.005 07
Face cleanser	0.000 352	0.003 52
Face cream, applied twice daily	0.001 13	0.003 72 (using 0.33% BHA, 95th percentile)
Body lotion, applied twice daily	0.0113	0.0372 (using 0.33% BHA, 95th percentile)
Shampoo	0.001	0.004 32 (using 0.43% BHA, 95th percentile)
Total	0.02	0.06

[1] Average intake assumes that the BHA content is 0.1%. The majority of products were in this category. Maximum intake assumes that the BHA content is 1%, unless otherwise specified. Dermal absorption is 5% (Schumann 1991).

[2] Shampoo assumed to be used on alternate days.

[3] Other products used on a daily basis include eye shadow, mascara and eyeliner. Their contribution to total intake is negligible. Oral exposure from lipstick is negligible in comparison with exposure from food (Appendix 1).

Confidence in the database on exposure to BHA is high. Information was available on concentrations of BHA in a large number of personal care products available in Canada. Such products are potentially the greatest source of exposure to BHA.

Health Effects Assessment

An overview of the reported health effects of BHA in laboratory animals is presented in Appendix 3.

BHA has been classified as possibly carcinogenic to humans (2B) based upon inadequate evidence in humans and sufficient evidence in experimental animals (IARC 1986, 1987) and as reasonably anticipated to be a carcinogen in the US National Toxicology Program Report on Carcinogens, starting with the sixth annual report in 1991 (NTP 1991) and continuing to the 11th report in 2005 (NTP 2005). IARC (2003) concluded that the mechanism by which BHA induced carcinogenicity in experimental animals was not relevant to humans. BHA is recognized by the US Food and Drug Administration as GRAS for use in food when the total antioxidant content is not greater than 0.02% of the fat or oil content (NTP 2005), and the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) allocated an ADI for humans of 0–0.5 mg/kg-bw at its 33rd meeting in 1989 (WHO 1989). BHA is permitted for use in food in Canada by virtue of its listing in Division 16 of the *Food and Drug Regulations* (Canada [1978]).

Most orally administered BHA is rapidly excreted in the urine, feces and expired air of rats (reviewed in Ito and Hirose 1987). The major metabolites are *O*-glucuronide and *O*-sulfate conjugates, as well as TBHQ, produced by *O*-demethylation. TBHQ in turn can be conjugated with glucuronide or sulfate and, in addition, undergoes redox cycling with the corresponding quinone, TBQ (*tert*-butyl quinone), to produce reactive oxygen species (evaluated by WHO 1998). Species variations in the proportion of the dose eliminated in urine or feces and in the preponderance of glucuronide or sulfate conjugates of BHA and the presence of conjugates of TBHQ in the urine were noted (Conning and Phillips 1986), with the pattern of elimination in humans being more similar to that in rats and rabbits than to that in dogs.

Treatment of male Sprague-Dawley rats for 3 days with 0.75% BHA in the diet resulted in a 40-fold increase in the level of hepatic messenger ribonucleic acid (mRNA) encoding for the uridine 5′-diphosphate (UDP)-glucuronyl transferase isoenzyme UGT1*06, whereas little effect on the expression levels of mRNA for cytochrome P450 enzymes was noted (Buetler et al. 1995). Administration of 0.75% BHA in the diet of female CD-1 mice for 3 weeks increased liver microsomal glucuronidation of estradiol and estrone by 2- and 3-fold, respectively (Zhu et al. 1997). Glucuronidation capacity of small intestinal cells was also increased in female Swiss Webster mice given 1% BHA in the diet for 10 days (Hjelle et al. 1985).

The toxicological profile of ingested BHA available prior to 1982 did not raise any concerns about its short- or long-term toxicity or its genotoxicity. None of the available long-term studies and only one of the short-term studies was conducted subsequent to the mid-1960s; consequently, these studies pre-dated the introduction more than a decade later of Good Laboratory Practice and standardized study protocols. Some of the long-

term studies incorporated reproductive segments into their protocols. Most of these studies did not test up to a maximum tolerated dose (MTD), and few histopathological observations were made in dietary feeding studies. The exception was the establishment of 2% BHA in the diet of rats as the MTD (Wilder and Kraybill 1948) and the finding of liver cell degeneration in weanling dogs fed BHA at 250 mg/kg-bw per day in their diet for 15 months (Wilder et al. 1960).

The first study reporting carcinogenic activity of BHA (Ito et al. 1982, 1983a) was conducted in male and female F344 rats fed BHA incorporated into pelletized diet at concentrations of 0%, 0.5% or 2%. One analysis showed that, in fact, processing had reduced the concentrations of BHA closer to 0.25% and 1%, but a subsequent study showed that no BHA was lost during the processing (reported in WHO 1983). Besides reduced body weight gain at the high dose, this study reported a very significant increase in the incidence of forestomach hyperplasia and neoplasia (papillomas and squamous cell carcinomas) in the treated animals, which showed a clear dose–response relationship. There was no significant difference in the incidence of tumours in any of the other organs. Bile duct proliferation, present in all groups, was reduced in a dose-related manner in the BHA groups. A subsequent review of the pathology slides suggested that neoplasms were present only in the forestomach of the high-dose animals.

Three other published carcinogenicity studies testing the effects of BHA in the male F344 rat are available (Ito et al. 1986; Masui et al. 1986; Williams et al. 1990b). All of these studies reported statistically significant incidences of papillomas of the forestomach at dietary concentrations of 1% BHA or higher and squamous cell carcinomas of the forestomach at dietary concentrations of 2% BHA. The Ito et al. (1986) study established a no-observed-effect level (NOEL) of 0.125% dietary BHA in the diet (measured as 0.11%, equivalent to 55 mg/kg-bw per day) for hyperplasia of the forestomach and a no-observed-adverse-effect level (NOAEL) of 0.5% BHA (measured as 0.47%, equivalent to 230 mg/kg-bw per day) for induction of papillomas of the forestomach.

In 2-year studies in other rodent species, forestomach papillomas and squamous cell carcinomas were induced in male B6C3F1 mice at concentrations of 0.5% and 1% in the diet and in male Syrian golden hamsters at concentrations of 1% and 2% in the diet (Masui et al. 1986). Ito et al. (1983b) found that 1% or 2% BHA in the diet of male Syrian golden hamsters induced 100% forestomach papillomas by 24 weeks (5.5 months).

No tumours of the analogous esophageal tissue or glandular stomach tissue were observed in carcinogenicity studies conducted in species lacking a forestomach. Guinea pigs fed a diet containing 1% BHA for 20 months did not exhibit any gross changes in the stomach (Ito and Hirose 1987).

Following the publication of the first carcinogenicity study in rats identifying the forestomach of rats as the primary target organ of BHA, numerous short-term studies were conducted in mice, rats and hamsters to characterize this carcinogenic effect of BHA. Observations on development and characteristics of preneoplastic lesions, labelling

index of the cells of the forestomach, and time course and reversibility of proliferative lesions of the forestomach were reported (WHO 1987, 1989). Studies by Altmann et al. (1985) concluded that BHA forestomach toxicity was due to localized irritancy and was not a systemic effect. Animals that were dosed with BHA via gavage demonstrated hyperplastic changes at the apex of the forestomach, while dietary administration produced hyperplasia in the area of the limiting ridge. This evidence supports the hypothesis that BHA-induced forestomach lesions are the result of localized tissue irritation, damage and cell turnover and suggests that BHA would demonstrate route-specific toxicity.

Short-term studies were also conducted in species without a forestomach (i.e., dogs, pigs, monkeys). In none of these studies was the extensive proliferative response elicited by BHA in the rodent forestomach observed in analogous tissues.

In separate studies, male and female Beagle dogs were fed 0%, 1.0% or 1.3% BHA (actual doses not calculated; Ikeda et al. 1986) or 0%, 0.25%, 0.5% or 1% BHA (equal to 0, 54, 111 and 219 mg/kg-bw per day in males and 0, 62, 112 and 231 mg/kg-bw per day in females) (Tobe et al. 1986) in the diet for 180 days (26 weeks). Body weight was reduced from controls at all dose levels, showing statistical significance only at the highest dose (1.3% of the diet). Both studies reported significant dose-related increases in liver weights of both male and female dogs in all of the treated groups, accompanied by histological changes described as fatty change and swelling or hypertrophy, as well as proliferation of the smooth endoplasmic reticulum, observed using electron microscopy (Ikeda et al. 1986). This is consistent with the increased activities of hepatic enzymes (aminopyrene demethylase, UDP-glucuronyl transferase, glutathione *S*-transferase and epoxide hydratase) and increased concentrations of cytochrome P450, cytochrome c reductase and cytochrome b also observed in this study. Thyroid organ weights were also statistically significantly increased over controls at both treatment levels with the exception of the high-dose females (Ikeda et al. 1986). No significant histological alteration of the thyroid was noted (Tobe et al. 1986). Neither study showed proliferative/hyperplastic lesions of the lower esophagus or stomach epithelium. The mitotic index of the basal layer of the esophageal epithelium showed no changes from control (Tobe et al. 1986). The lowest-observed-effect level (LOEL) in the dog, based on liver weight increases and histopathological changes, was 0.25%, equal to 54 mg/kg-bw per day.

Because of the interest in the effect of BHA on the forestomach of rodents, preserved stomach tissue samples from a 16-week embryotoxicity study conducted with BHA in female Danish Landrace pigs (Hansen et al. 1982) were evaluated after the fact (Olsen 1983; Würtzen and Olsen 1986). In this study, BHA was incorporated into the diet of groups of 9–13 female pigs at 0, 50, 200 or 400 mg/kg-bw per day (corresponding to levels of 0%, 0.5%, 1.9% or 3.7% in the diet) starting on the first day of mating and extending to day 110 of gestation. Significantly lower body weight gain was noted in the dams in the high dose group (400 mg/kg-bw per day), and statistically significant, dose-related increases in the absolute and relative weights of the liver and thyroid were observed in all treated groups. No histopathological changes were reported in the liver,

whereas large follicles with flattened epithelium in the thyroid were noted in some animals, particularly in the high-dose group. There were epithelial changes in the esophageal portion of the stomach of both control and treated pigs, a common spontaneous lesion in pigs. In addition, linear yellow-brown rough epithelium was seen along the entire length of the esophagus in a few pigs in the middle and high dose groups. Proliferative and parakeratotic changes of the esophageal epithelium were confirmed in these treated animals by microscopic examination. There was no effect of treatment with BHA on reproductive parameters, and there was no consistent sign of a teratological effect attributable to BHA. Based on the elevated absolute and relative liver and thyroid weights and histopathological changes in the thyroid gland at all treatment levels, the LOEL for this study was 50 mg/kg-bw per day (Hansen et al. 1982; Olsen 1983; Würtzen and Olsen 1986).

Female cynomolgus monkeys were administered BHA by corn oil gavage 5 days/week for 12 weeks at initial doses of 0, 125 or 500 mg/kg-bw per day (Iverson et al. 1986). Due to the frequent observation of vomiting in the high dose group after gavage, the dose was reduced to 250 mg/kg-bw per day after 20 days. No lesions were attributable to treatment with BHA either by gastroscopy during the study or at necropsy. In particular, histological examination of stomach epithelial tissue showed no unusual proliferation or hyperplasia of epithelial layers and no thickening of epithelial layers. However, the mitotic index of 5000 cells evaluated from the basal cell layer of the squamous epithelium in sections of esophagus taken 5 mm from the cardia was increased about 2-fold in the high dose group. Liver weights were increased in a dose-related manner at both treatment levels both as the absolute value and relative to body weight, and electron microscopy of liver samples revealed cytomegaly but no consistent increase in smooth endoplasmic reticulum. BHA treatment had no effect on the activity of the hepatic microsomal mono-oxygenases aminopyrene *N*-demethylase, acetanilide hydroxylase or ethoxycoumarin deethylase, on cytosolic glutathione transferase activities towards aryl, aralkyl or epoxy substrates, or on concentration of hepatic cytochrome P450. A decrease in the activity of ethoxyresorufin deethylase was observed in the high-dose animals. UDP-glucuronyl transferase activity was not assessed. The LOEL for this study was 125 mg/kg-bw per day, based on increased liver weights.

BHA has been shown to induce forestomach tumours in rodents following oral administration. However, no evidence of carcinogenicity was observed when BHA was administered to mice by subcutaneous injection, intraperitoneal injection or topical application (Busch 1984; NTP 2005). In a study by Stoner et al. (1973), a total dose of 6000 mg/kg-bw was administered by intraperitoneal injection to groups of A/He mice over a period of 8 weeks. The achieved dose was calculated to be 107 mg/kg-bw per day. The investigators grossly examined liver, spleen, thymus, intestine, and salivary and endocrine glands, while lungs were examined microscopically. Gross lesions in these tissues were also examined microscopically. While the study was limited in duration and in endpoints examined, there were no gross or histopathological lesions reported for the BHA-treated animals. In a tumour promotion assay (Berry et al. 1978), groups of 30 CD-1 mice were dermally exposed to BHA twice a week for 30 weeks. The applications of 1 mg/event were calculated to be equivalent to daily application rates of 10–15 mg/kg-bw

per day. The animals were initially challenged with a tumour initiator (7,12-dimethylbenz[a]anthracene), and the assay was designed to test the tumour promotion ability of BHA. After 30 weeks, histopathological examinations revealed no evidence of pathology in the BHA-treated animals.

The toxicity noted in rodent forestomach following oral dosing was not noted in C3H/Anf mice following lifetime dermal exposure. Groups of 50 mice were dermally treated with 0.10 or 10 mg of BHA per week for the duration of their life (Hodge et al. 1966). Since body weights were not provided in this study, a range of doses was calculated by the reviewer to represent the dosage applied to animals with a typical range of adult body weights. The 10 mg dose was determined to be equivalent to 48–100 mg/kg-bw per day for males and 57–133 mg/kg-bw per day (NOEL) for females. The parameters measured were body weight, survival, gross necropsy of each mouse after its preservation in formalin and histopathological examination of skin where autolysis had not occurred. There were no treatment-related observations of lesions from the gross necropsy or histopathological evaluation of skin, while isolated incidences of skin lesions (attributed to weekly clipping of the fur) were noted. While the examinations of animals were limited in scope, this study (Hodge et al. 1966) demonstrated a lack of systemic toxicity following long-term dermal exposure of rodents to BHA. The lack of reported lesions in studies of BHA toxicity via dermal, intraperitoneal and subcutaneous routes of exposure might be the result of limited study design. However, it has been suggested that the lesions noted in the oral feeding studies of BHA are a local, tissue-specific toxicity and are not a systemic effect (Altmann et al. 1985).

Numerous *in vitro* mutagenicity assays of BHA have been conducted (reviewed in Williams et al. 1999) and include reverse mutation assays in strains of *Salmonella typhimurium*, mutation at the HGPRT locus in mammalian cells, and chromosomal aberration assays and sister chromatid exchange assays in various Chinese hamster cell cultures. Only the *in vitro* chromosomal aberration studies in the presence of metabolic activation showed consistent positive results, while the outcome of the vast majority of the other studies was negative. Studies of clastogenic potential conducted *in vivo* were not available for BHA. However, negative results were found for other endpoints of deoxyribonucleic acid (DNA) interaction conducted *in vivo* in hepatocytes and forestomach of the F344 rat, including DNA adduct formation, the majority of studies assessing DNA strand breaks and repair, and one study assessing induction of unscheduled DNA synthesis. A single comet assay conducted in mice showed positive results in stomach and colon (Sasaki et al. 2002). In addition, the results from the assessment of the clastogenic potential of TBHQ, an oxidative metabolite of BHA, are relevant and of interest. The results of *in vitro* chromosomal aberrations were also uniformly positive, whether in the absence or presence of metabolic activation. However, TBHQ did not induce chromosomal aberrations or micronucleus formation in well-conducted *in vivo* studies (evaluated in WHO 1998).

Numerous studies have been conducted on the effects of BHA on the potentiation or inhibition of carcinogenicity of the colon, forestomach, urinary bladder, stomach, liver, lung, mammary gland and pancreas, as well as perinatal carcinogenicity, induced by a

variety of clearly carcinogenic compounds. The totality of evidence suggests that BHA potentiates, inhibits or has no effect on the ability of carcinogens to act, depending on the exact chemical being tested and the circumstances under which the test was conducted (WHO 1987, 1989).

JECFA had consistently requested a multigeneration reproduction study with BHA and, at its last evaluation of BHA (33rd meeting; WHO 1989), had indicated that one was ongoing. However, an ADI of 0–0.5 mg/kg-bw, based on data from a chronic rat feeding study, was allocated at this meeting in the absence of this study. The only new reproduction study, a one-generation study in rats (Jeong et al. 2005) published 16 years later, was not likely the study referred to. In addition to the study by Jeong et al. (2005), four studies evaluated the developmental toxicity of BHA in the mouse (Stokes and Scudder 1974), rat (Vorhees et al. 1981), rabbit (Hansen and Meyer 1978) and pig (Hansen et al. 1982).

Jeong et al. (2005) conducted a one-generation reproduction study in Sprague-Dawley rats with BHA doses of 0, 10, 100 or 500 mg/kg-bw per day, administered by corn oil gavage. Besides terminating the study when the F_1 generation reached 13 weeks of age, rather than producing an F_2 generation, other departures from standard protocol for a study of reproductive toxicity included a 2-week rather than a 10-week pre-mating period and assessment of sperm morphology by measuring the sperm heads of dried preparations from 10 sperm per animal, rather than assessing sperm abnormalities from wet preparations of a minimum 200 sperm per animal. Small, but statistically significant, differences from control groups were reported for numerous parameters at the highest BHA dose, 500 mg/kg-bw per day, which included decreased mating ratio and increased cohabitation time to mating of the F_0 rats; decreased serum thyroxine and testosterone levels in F_0 male rats after 7 weeks of treatment; organ weight changes expressed as the absolute and/or relative weights of liver, adrenal and thyroid (increases), spleen, ventral prostate, testes and vagina (decreases) variously in F_0 and F_1 adults; decreased postnatal body weight gain of both male and female F_1 pups; delays in the appearance of vaginal patency and preputial separation; decreased absolute and relative liver weights in the female F_1 weanling rats; and decreased sperm count in the cauda epididymis of F_1 rats exposed for 13 weeks, as well as decreases in several sperm motility parameters and decreased serum testosterone levels in this group. There were very few observations of treatment-related effects at the lower doses, some related to differences in organ weights with no accompanying histopathological observations or to sperm head measurements using questionable techniques. The observation in both the 100 and 500 mg/kg-bw per day treated groups of histopathological changes to thyroid follicular epithelial cells in both male and female adult F_1 rats and decreases in serum testosterone concentrations in F_0 males at 7 weeks of treatment suggests that a dose of 100 mg/kg-bw per day was the LOEL for endocrine effects in this study. The authors cited observations from other studies that hypothyroidism induced during the early neonatal period through to weaning results in delays in sexual maturation, testicular atrophy, impaired accessory reproductive organ development, reduced gonadotrophins and inhibition of steroidogenesis.

Vorhees et al. (1981) conducted a study in Sprague-Dawley rats with a similar protocol to Jeong et al. (2005), with the exception that BHA was administered in the diet instead of by gavage and with more emphasis placed on behavioural testing rather than reproductive or endocrine and pathology parameters. The dose range from the diet for adult rats prior to breeding and during lactation was similar to that for the top two dose groups in the Jeong et al. (2005) study: 0%, 0.125%, 0.25% and 0.5%, equal to an average of 0, 100, 200 and 400 mg/kg-bw per day, respectively; however, doses received by lactating rats (reported by the authors) were double, and those for the weanling rats would also be higher. BHA treatment had no effect on reproductive parameters, and minimal effects were noted in the extensive behavioural testing. Delayed startle development was noted at the two highest concentrations; development of vaginal patency was not affected at any dietary concentration of BHA. Growth retardation was noted in the pups on days 14 and 21 of the lactation period at the highest dietary concentration (0.5%). In addition, mortality was increased up to postnatal day 30 in this group and marginally at the 0.25% dietary concentration. The NOEL for reproductive toxicity in the study was 0.125% BHA in the diet, equal to 100 mg/kg-bw per day.

No adverse effects were detected in a developmental toxicity study in rabbits using BHA doses of 0, 50, 200 or 400 mg/kg-bw per day administered by gavage on days 7–18 of gestation (Hansen and Meyer 1978). It should be noted that the administration period does not meet present-day requirements (days 6–29 of gestation in rabbits).

The same research group (Hansen et al. 1982) conducted an embryotoxicity study in Danish Landrace pigs in which the pigs received BHA doses of 0, 50, 200 or 400 mg/kg-bw per day in the diet from the day of artificial insemination to day 110 of gestation. No effects on reproductive or developmental parameters were noted. Results related to organ weight determinations and histopathology have been described above.

A single epidemiological study conducted as part of the Netherlands Cohort Study did not find an association between the usually low intakes of BHA from its presence in food (about 100 µg/day) and stomach cancer (Botterweck et al. 2000). Rather, there was an inverse trend in relative risk with higher intakes of BHA, which was not statistically significant.

Confidence in the toxicity database for BHA is moderate. While numerous chronic, genotoxicity and reproductive toxicity studies were identified, there exists some uncertainty in whether the existing database is adequate to fully characterize toxicity via all routes and durations of exposure.

Characterization of Risk to Human Health

Based upon information provided in studies from the published literature on BHA and evaluations by JECFA, two critical effects were identified for characterization of risk to human health following oral dosing. The first was carcinogenesis of the squamous epithelium of the forestomach in rodents following lifetime feeding, and the second was changes in endocrine measures secondary to enzyme induction by BHA. These secondary

effects primarily influenced the thyroid gland but also altered sex hormones and the adrenal gland at higher doses following subchronic oral dosing.

Two dermal toxicity studies of limited protocol and reporting were identified for BHA. However, in the lifetime toxicity study (Hodge et al. 1966), BHA did not induce any notable systemic toxicity in the mouse. Similarly, dermal toxicity was limited to sporadic indications of ulceration, which were attributed to clipping injury. There were no indications of hyperplasia or other preneoplastic lesions in the dermis, indicating that the site of contact via the dermal route was not susceptible to cytotoxicity and cell turnover at the dose tested. The lack of systemic toxicity in these limited dermal studies was also reported in a short-term (8 weeks) intraperitoneal study dosing animals up to 107 mg/kg-bw per day (Stoner et al. 1973). Some authors suggest that lesions noted in feeding studies are local and not systemic effects, and the studies conducted by intraperitoneal, subcutaneous and dermal routes, while limited in detail, support this conclusion (Altmann et al. 1985).

BHA added to the diet of rats, mice and hamsters induced hyperplasia and neoplasia of the squamous epithelium of the forestomach. However, there was no evidence in short-term studies of the extensive proliferative response in analogous tissues of species lacking a forestomach (dogs, pigs and monkeys) or of tumours of the analogous esophageal tissue or glandular stomach tissue in longer-term studies in guinea pigs. Based on the study of Ito et al. (1986), the NOEL for hyperplasia of the forestomach epithelium in the rat, a reversible lesion, was 0.125% of the diet, equivalent to 55 mg/kg-bw per day; the NOAEL for neoplasia in this study was 0.5% of the diet, equivalent to 230 mg/kg-bw per day.

The weight of evidence from the available data suggests that BHA is not genotoxic. While the results of *in vitro* chromosomal aberration assays showed consistently positive results, the vast majority of studies assessing *in vitro* point mutation and *in vivo* DNA adduct formation, induction of unscheduled DNA synthesis and strand breaks and repair in hepatocytes and forestomach of the rat were negative. Studies assessing the potential for induction of chromosomal aberrations *in vivo* were not available for BHA, but there were several mouse micronucleus assays as a measure of clastogenic potential that were conducted with TBHQ, the principal oxidative metabolite of BHA and another antioxidant food additive. As for BHA, results from *in vitro* chromosomal aberration studies with TBHQ were positive, while the results of well-conducted mouse micronucleus assays as an *in vivo* measure of clastogenic potential were negative (evaluated in WHO 1998).

The database indicates that BHA induces cellular damage, cell turnover and the eventual development of tumours in the forestomach—an anatomical structure for which there is no analogous counterpart in humans or other mammalian, non-rodent species—in rodents following oral exposure. The lesions were limited to the site of initial stomach contact, as indicated by studies dosing animals via different oral routes (diet or gavage). The weight of evidence suggests that BHA is not directly genotoxic, since it did not form DNA

adducts in the liver or forestomach tissue, and there was no significant association between the usually low intake of BHA from food additive use and stomach cancer in a human cohort of 3500 subjects. A comparison of the NOAEL for tumour formation (230 mg/kg-bw per day) with the highest mean BHA exposure of 0.095 mg/kg-bw per day in 4- to 8-year-old children produces an adequate margin of exposure of greater than 2400. While a similar site-limited, irritation response could occur in humans, excessively high doses would be required to generate such a localized irritation response. The initial toxicological sequelae (hyperplasia, inflammation) were noted in the animal database to be fully reversible events.

BHA has been shown to induce liver and intestinal UDP-glucuronyl transferase, which mediates the conjugation of both BHA and its metabolite TBHQ with glucuronide. This increased glucuronidation capacity likely contributes to its anticarcinogenic effect through increased inactivation of carcinogens by conjugation. In addition, the effects on a variety of organs can be attributed to increased clearance of hormones resulting from enhanced liver enzyme activity. The LOEL for non-cancer effects was found to be 50 mg/kg-bw per day in the pig, the lowest dose tested, based on increased thyroid and liver weights without effects on reproductive/developmental parameters (Hansen et al. 1982).

Comparison of the lowest critical non-neoplastic effect level secondary to induction of UDP-glucuronyl transferase in the liver and small intestine (50 mg/kg-bw per day) with the highest mean BHA exposure of 0.095 mg/kg-bw per day in 4- to 8-year-old children indicates a margin of exposure greater than 500. Since the LOEL is based on a threshold for induction of a compensatory liver enzyme, this margin of exposure would be considered adequate to protect human health.

A lifetime dermal toxicity study in the mouse (Hodge et al. 1966) demonstrated no adverse systemic effects over an estimated dose range of 48–100 mg/kg-bw per day for males and 57–133 mg/kg-bw per day for females (NOELs).While the examination was limited in scope, no localized toxicity was observed. Although detailed necropsies were not performed on these animals, there were no indications of systemic toxicity reported in treated mice, and survival rates were not adversely affected. Furthermore, a second dermal toxicity study similarly reported an absence of toxicity up to a dose level of 10–15 mg/kg-bw per day for 30 weeks (Berry et al. 1978). There was a lack of toxicity noted in an 8-week intraperitoneal injection mouse assay up to a dose level of 107 mg/kg-bw per day (Stoner et al. 1973). This study examined a variety of organs and found no indications of systemic toxicity. However, since the dermal and intraperitoneal studies did not include extensive histopathology examinations, it is uncertain whether effects observed following oral dosing could occur following dermal exposure. Thus, endpoints observed in the oral toxicity database were considered to be appropriate for characterizing risk following dermal exposure.

The combined dermal intake of BHA resulting from frequently used personal care products has been estimated for several age groups in the population. The “average” (see Table 8) predicted intakes ranged from 0.004 mg/kg-bw per day (for infants) to 0.02 mg/kg-bw per day (for women and children aged 6 months to 12 years). Comparison of

these intakes with the NOAEL for tumour formation (230 mg/kg-bw per day) results in margins of exposure of 57 500 and 11 500, respectively. The highest predicted intakes ranged from 0.01 mg/kg-bw per day (for men) to 0.07 mg/kg-bw per day (for children aged 6 months to <5 years). Comparisons of these intakes with the NOAEL for tumour formation results in margins of exposure of 23 000 and 3300, respectively.

The lowest critical effect level for non-cancer endpoints was 50 mg/kg-bw per day, based upon increased liver and thyroid weights. Comparison of this value with the estimates of 0.004 mg/kg-bw per day (for infants) and 0.02 mg/kg-bw per day (for women and children aged 6 months to 12 years) results in margins of 12 500 and 2500, respectively. Similarly, comparison of the highest estimated intakes of 0.01 mg/kg-bw per day (for men) and 0.07 mg/kg-bw per day (children aged 6 months to <5 years) with the lowest critical effect level for non-cancer endpoints results in margins of exposure of 5000 and 700, respectively.

The lack of systemic toxicity noted in the limited dermal studies was also noted in studies conducted by intraperitoneal and subcutaneous routes. While these studies were of limited quality, collectively, they indicate a lack of systemic toxicity by non-oral routes of exposure. Since no toxicity was observed following long-term dermal exposure, the use of oral toxicity endpoints is considered very conservative, and the derived margins of exposure are considered adequately protective for human health.

Uncertainties in Evaluation of Risk to Human Health

Although some food categories that would contribute minor amounts to BHA exposure were not included in the assessment, dietary exposure to BHA may have been overestimated, as insufficient quantitative information was available to account for the following factors: actual use levels of BHA in relation to the maximum permitted levels of use specified in the *Food and Drug Regulations*; actual use levels of BHA in combination with other antioxidants; possible discontinued use of BHA by manufacturers in some foods in which it is permitted; and possible losses of BHA in foods during processing, transport and storage.

Only limited information was available from which to estimate dermal absorption of BHA in humans. Better characterization of absorption would reduce uncertainty in the estimates of intake from personal care products. There is some uncertainty with the characterization of toxicity following dermal exposure. Studies of long-term dermal administration were limited to older reports with limited examination of endpoints. However, there was a consistent absence of observations of adverse effects following subcutaneous, intraperitoneal or dermal administration. The use of oral toxicity endpoints to characterize risk following dermal exposure is considered very conservative. Additional studies to further characterize toxicity via the dermal route would reduce uncertainties in the assessment.

Conclusion

Based on the information presented in this screening assessment, it is concluded that BHA 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 or its biological diversity or that constitutes or may constitute a danger to the environment on which life depends. Additionally, BHA meets the persistence criteria but does not meet the criteria for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Based on the available information on its potential to cause harm to human health, it is concluded that BHA is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore concluded that BHA does not meet any of the criteria under section 64 of CEPA 1999.

This substance will be considered for inclusion in the Domestic Substances List inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

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Appendix 1. Estimated dietary exposure to BHA from food additive use[1,2,3,4]

Age group (years)	Sex	Mean BHA exposure (mg/kg-bw per day)
<1	Male and female	0.023
1–3	Male and female	0.088
4–8	Male and female	0.095
9–13	Male	0.071
	Female	0.062
14–18	Male	0.052
	Female	0.046
19–30	Male	0.042
	Female	0.035
31–50	Male	0.033
	Female	0.033
51–70	Male	0.028
	Female	0.029
71+	Male	0.026
	Female	0.026

[1] Food intake data were obtained from the Canadian Community Health Survey (CCHS) – Cycle 2.2 on Nutrition (Statistics Canada 2004). One-day intakes were employed, and no adjustments were made to account for usual daily intakes, a statistical adjustment that approximates the distribution of long-term average daily exposure. Exposure figures were adjusted for body weight. Body weights are not reported by the CCHS for children less than 2 years of age; hence, values derived from the Continuing Survey of Food Intakes by Individuals (CSFII 1994–96, 1998) were used.

[2] The foods that are permitted to contain BHA as per Division 16, Table XI, of the *Food and Drug Regulations* include those that make a significant contribution to the diet and/or are popular foods for children. Foods that are not widely consumed or for which intake data were not available were not included in the exposure assessment.

[3] Actual use levels of BHA were available for a few foods that were considered in the assessment. In all other applicable cases, the BHA concentration in food was assumed to be a fraction (one-half, one-third or one-quarter, depending on the number of antioxidants permitted) of the maximum permitted use level of antioxidants with which BHA may be used in combination (i.e., butylated hydroxytoluene, propyl gallate, TBHQ).

[4] Dietary exposure to BHA may have been overestimated, as insufficient quantitative information was available to account for the following factors:

- actual use levels of BHA in relation to the maximum permitted levels of use specified in the *Food and Drug Regulations*;
- actual use levels of BHA in combination with other antioxidants;
- possible discontinued use of BHA by manufacturers in some foods in which it is permitted; and
- possible losses of BHA in foods during processing, transport and storage.

Appendix 2. Upper-bounding estimates of exposure to BHA in personal care products, based upon ConsExpo version 4.1 (ConsExpo 2006)

Type of product	Personal care product scenario in ConsExpo	Assumptions[1]	Predicted intake (mg/kg-bw per day)
Baby product	Baby cream	Exposure frequency: twice daily Body weight: 7.5 kg (Health Canada 1998) Exposed area: 201 cm^2 Applied amount: 0.27 g	
		Concentration of BHA: 0.1%	Chronic: 0.0036
		Concentration of BHA: 1%	Chronic: 0.036
Deodorant	Deodorant spray	Exposure frequency: twice daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 100 cm^2 Applied amount: 2.6 g	
		Concentration of BHA: 0.1% (all deodorants are in 0–0.1% category)	Chronic: 0.003 66
Eye makeup	Eye shadow	Exposure frequency: twice daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 24 cm^2 Applied amount: 0.01 g	
		Concentration of BHA: 0.1%	Chronic: 1.41E-5
		Concentration of BHA: 1%	Chronic: 0.000 141
	Mascara	Exposure frequency: once daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 1.6 cm^2 Applied amount: 0.025 g	
		Concentration of BHA: 0.1%	Chronic: 1.76E-5
		Concentration of BHA: 1%	Chronic: 0.000 176
	Eyeliner	Exposure frequency: once daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 3.2 cm^2 Applied amount: 0.005 g	
		Concentration of BHA: 0.1%	Chronic: 3.52E-6
		Concentration of BHA: 1%	Chronic: 3.52E-5
Face makeup	Facial makeup	Exposure frequency: once daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 638 cm^2 Applied amount: 0.8 g	
		Concentration of BHA: 0.1%	Chronic: 0.000 564
		Concentration of BHA: 1%	Chronic: 0.005 64

Type of product	**Personal care product scenario in ConsExpo**	**Assumptions[1]**	**Predicted intake (mg/kg-bw per day)**
Fragrance	Eau de toilette	Exposure frequency: ~3 times daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 200 cm^2 Applied amount: 0.61 g	
		Concentration of BHA: 0.1%	Chronic: 0.001 28
Hair grooming	Hairspray	Exposure frequency: more than once a day; 438 times a year Body weight: 70.9 kg (Health Canada 1998) Exposed area: 638 cm^2 Applied amount: 0.6 g	
		Concentration of BHA: 0.1%	Chronic: 0.000 507
		Concentration of BHA: 1%	Chronic: 0.005 07
	Hair gel	Exposure frequency: daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 638 cm^2 Applied amount: 0.3 g	
		Concentration of BHA: 0.1%	Chronic: 0.000 207
		Concentration of BHA: 1%	Chronic: 0.002 07
Hair shampoo, adult		Exposure frequency: 260 times a year Body weight: 70.9 kg (Health Canada 1998) Exposed area: 1.55×10^3 cm^2 Applied amount: 20 g	
		Concentration of BHA: 0.1% Retention factor of 10% to account for shampoo that is washed off	Chronic: 0.001
		Concentration of BHA: 0.43% (95th percentile) Retention factor of 10% to account for shampoo that is washed off	Chronic: 0.004 32
Hair shampoo, age 6 months to <5 years		Exposure frequency: 156 times a year (SDA 2005) Body weight: 13 kg (Health Canada 1998) Exposed area: 435 cm^2 (Health Canada 1995) Applied amount: 14 g (derived from Health Canada 1995)	
		Concentration of BHA: 0.1% Retention factor of 10% to account for shampoo that is washed off	Chronic: 0.0023

Type of product	**Personal care product scenario in ConsExpo**	**Assumptions[1]**	**Predicted intake (mg/kg-bw per day)**
		Concentration of BHA: 0.43% (95th percentile) Retention factor of 10% to account for shampoo that is washed off	Chronic: 0.009 89
Hair shampoo, age 5–12 years		Exposure frequency: 156 times a year (SDA 2005) Body weight: 27 kg (Health Canada 1998) Exposed area: 605 cm^2 (Health Canada 1995) Applied amount: 19 g (derived from Health Canada 1995)	
		Concentration of BHA: 0.1% Retention factor of 10% to account for shampoo that is washed off	Chronic: 0.0015
		Concentration of BHA: 0.43% (95th percentile) Retention factor of 10% to account for shampoo that is washed off	Chronic: 0.006 46
Lipstick		Exposure frequency: 4 times daily Body weight: 70.9 kg (Health Canada 1998) Amount ingested: 0.01 g	
		Concentration of BHA: 0.1%	Chronic: 0.000 564
		Concentration of BHA: 1%	Chronic: 0.005 64
Shaving preparation; all are in the 0–0.1% category	Shaving cream	Exposure frequency: once daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 319 cm^2 Applied amount: 2 g	
		Concentration of BHA: 0.1% Applied retention factor of 10% to account for washing	Chronic: 0.000 141
	Aftershave	Exposure frequency: once daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 319 cm^2 Applied amount: 1.2 g	
		Concentration of BHA: 0.1%	Chronic: 0.000 846
Skin cleanser	Face cleanser	Exposure frequency: twice daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 638 cm^2 Applied amount: 2.5 g	

Type of product	**Personal care product scenario in ConsExpo**	**Assumptions**[1]	**Predicted intake (mg/kg-bw per day)**
		Concentration of BHA: 0.1% Applied retention factor of 10% to account for washing	Chronic: 0.000 352
		Concentration of BHA: 1% Applied retention factor of 10% to account for washing	Chronic: 0.003 52
Skin moisturizer	Face cream	Exposure frequency: twice daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 638 cm^2 Applied amount: 0.8 g	
		Concentration of BHA: 0.1%	Chronic: 0.001 13
		Concentration of BHA: 0.33% (95th percentile)	Chronic: 0.003 72
	Body lotion, adult	Exposure frequency: twice daily Body weight: 70.9 kg (Health Canada 1998) Exposed area: 1.69×10^4 cm^2 Applied amount: 8 g	
		Concentration of BHA: 0.1%	Chronic: 0.0113
		Concentration of BHA: 0.33% (95th percentile)	Chronic: 0.0372
	Body lotion, age 6 months to <5 years	Exposure frequency: twice daily Body weight: 13 kg (Health Canada 1998) Exposed area: 4910 cm^2 (derived from Health Canada 1995) Applied amount: 2.3 g (derived from Health Canada 1995)	
		Concentration of BHA: 0.1%	Chronic: 0.0177
		Concentration of BHA: 0.33% (95th percentile)	Chronic: 0.0583
	Body lotion, age 5–12 years	Exposure frequency: twice daily Body weight: 27 kg (Health Canada 1998) Exposed area: 8450 cm^2 (derived from Health Canada 1995) Applied amount: 4 g (derived from Health Canada 1995)	
		Concentration of BHA: 0.1%	Chronic: 0.0148
		Concentration of BHA: 0.33% (95th percentile)	Chronic: 0.0489

Type of product	**Personal care product scenario in ConsExpo**	**Assumptions**[1]	**Predicted intake (mg/kg-bw per day)**
Tanning preparation	Sunscreen lotion	Exposure frequency: 75 times/year (>2 months) Body weight: 70.9 kg (Health Canada 1998) Exposed area: 1.82×10^4 cm^2 Applied amount: 10 g	
		Concentration of BHA: 0.1%	Chronic: 0.001 45
		Concentration of BHA: 1%	Chronic: 0.0145

[1] From ConsExpo (2006) and Health Canada (1995, 1998) unless otherwise specified. All scenarios are for 5% absorption, based upon Schumann (1991), with the exception of lipstick (100% from oral exposure) It is noted that use of eau de toilette, hair spray and spray deodorant result in inhalation exposure, however this is negligible compared to the dermal exposure.

Appendix 3. Summary of health effects of BHA reported in animal studies

Endpoint	Lowest effect levels[1]/Results
Laboratory animals and *in vitro*	
Acute toxicity	**Oral LD_{50}** (rat) = 2200–5000 mg/kg-bw (Lehman et al. 1951; Bunnell et al. 1955). **Oral LD_{50}** (mouse) = 2000 mg/kg-bw (Lehman et al. 1951; Bunnell et al. 1955).
Short-term repeated-dose toxicity	**Oral LOEL** = 0.5% diet, equivalent to 500 mg/kg-bw per day, 4-week feeding study, male F344 rat, based upon hyperplastic forestomach lesions (Cantoreggi et al. 1993). **Dermal LOEL** = 8 g/kg-bw, inbred black guinea pigs, based upon ultrastructural morphological lesions of the epidermis, 20% BHA in lanolin, 6 weeks (Riley and Seal 1968). **Intraperitoneal NOAEL** = 6000 mg/kg (calculated by reviewer to be equivalent to 107 mg/kg-bw per day). BHA was administered by intraperitoneal injection to groups of A/He mice 3 times/week for a period of 8 weeks. The investigators grossly examined liver, spleen, thymus, intestine, and salivary and endocrine glands, while lungs were examined microscopically. Gross lesions in the examined tissues were also examined microscopically. There were no gross or histopathological lesions reported for the BHA-treated animals. [Additional studies: Berry et al. 1978; Ito et al. 1984; Nera et al. 1984; Altmann et al. 1985, 1986; Clayson et al. 1986; Hirose et al. 1987]
Subchronic toxicity	**Oral LOEL** = 0.125% diet, equivalent to 125 mg/kg-bw per day, male Wistar rat, 90-day feeding study, based upon mild hyperplasia of the forestomach; no NOEL for this study (Altmann et al. 1986). **Oral LOEL** = 0.25% diet, equal to 54 mg/kg-bw per day, Beagle dogs, 180-day feeding study, based on liver weight increases and histopathological changes resulting from enzyme induction; no histopathological alterations to thyroid; no proliferative/hyperplastic lesions of the lower esophagus or stomach epithelium; no NOEL for this study (Ikeda et al. 1986; Tobe et al. 1986). **Oral LOEL** = 0.5% diet, equal to 50 mg/kg-bw per day; 110-day feeding study in pregnant female Landrace pigs, based on increased liver and thyroid weights and histopathological changes in the thyroid; observations of rough epithelium of the esophagus in a few mid- and high-dose pigs; no NOEL for this study (Hansen et al. 1982; Olsen 1983; Würtzen and Olsen 1986). **Oral LOEL** = 125 mg/kg-bw per day, cynomolgus monkeys, 12-week feeding study, based upon dose-related increases in liver weights and liver cell hypertrophy; increased mitotic index in squamous epithelium of the distal esophagus at 250 mg/kg-bw per day; no NOEL for this study (Iverson et al. 1986).

Endpoint	Lowest effect levels[1]/Results
	[Additional studies: Ito et al. 1983b, 1986; Iverson et al. 1985; Altmann et al. 1986; Clayson et al. 1986; Newberne et al. 1986; Hirose et al. 1987a]
Developmental/ reproductive toxicity	**Oral NOEL** = 400 mg/kg-bw per day (diet), adult female pig (Danish Landrace), 131-day feeding study following artificial insemination; no effects on reproductive parameters at highest dose tested (Hansen et al. 1982). **Oral LOEL** = 100 mg/kg-bw per day (gavage), based on histopathological changes to thyroid follicular cells in F_1 male and female adult rats, decreased serum testosterone in F_0 male rats after 7 weeks; NOEL of 10 mg/kg-bw per day (Jeong et al. 2005). **Oral LOEL** = 200 mg/kg-bw per day (diet); minimal effects in behavioural testing; growth retardation during last week of lactation and increased mortality up to postnatal day 30 in F_0 pups (Vorhees et al. 1981). [Additional studies: Telford et al. 1962; Fabrizio 1974; Stokes and Scudder 1974; Hansen and Meyer 1978]
Chronic toxicity/ carcinogenicity	Male B6C3F1 mice, 0%, 0.5% or 1% diet, 104 weeks, significant increases in incidence of forestomach papillomas in both dosed groups; forestomach hyperplasia (0/39, 10/37**, 35/43**, ** $p < 0.001$); forestomach papillomas (0/39, 5/37*, 5/43*, * $p < 0.05$); squamous cell carcinomas (0/39, 1/37, 2/43) (Masui et al. 1986). Male Fischer 344 rats, 0%, 0.125%, 0.25%, 0.5%, 1.0% or 2.0% diet, 104 weeks; significantly (* $p < 0.01$; ** $p < 0.001$) increased incidence of hyperplasia of the forestomach (0/50, 1/50, 7/50*, 16/50**, 44/50**, 50/50**), forestomach papillomas (0/50, 0/50, 0/50, 0/50, 10/50*, 50/50**), forestomach squamous cell carcinomas (0/50, 0/50, 0/50, 0/50, 0/50, 11/50**); **NOEL** = 0.11% (nominally 0.125%), equivalent to 55 mg/kg-bw per day, based on hyperplasia (reversible); **NOAEL** = 0.47% (nominally 0.5%), equivalent to 230 mg/kg-bw per day, based on forestomach papillomas (Ito et al. 1986). Male Syrian golden hamsters, 0%, 1% or 2% diet, 104 weeks, significant (** $p < 0.001$) increases in incidence of forestomach papillomas in both dosed groups; forestomach hyperplasia (9/52, 53/55**, 40/40**); forestomach papillomas (0/52, 54/55**, 38/40**); squamous cell carcinomas (0/52, 4/55*, 4/40*, * $p < 0.05$) (Masui et al. 1986). C3H/Anf mice, 0.10 or 10 mg applied to the skin once per week for life. No evidence of localized or systemic effects noted in treated animals. Survival rates were comparable to controls. Lifetime achieved doses were calculated by the reviewer to be 48 to 100 and 57 to 133 mg/kg-bw per day (**NOEL**) for male and female mice, respectively (Hodge et al. 1966).

Endpoint	Lowest effect levels[1]/Results
	[Additional studies: Tomii and Aoki 1977; Ito et al. 1982, 1983a; Masui et al. 1986; Ito and Hirose 1987; Williams et al. 1990b]
Special studies on endocrine function	Immature rat uterotropic assay and Hershberger assay; **LOEL** = 50 mg/kg-bw per day by subcutaneous injection for 3 days, based on absolute and relative uterine weights in immature (20-day-old) female rats; effect was abolished by 17β-estradiol treatment; negligible effect on the androgenic activity in castrated male rats (Kang et al. 2005). Uterotropic assay, ovariectomized CD-1 mice; 18-day feeding 0.75% BHA in diet for 18 days (equivalent to 1125 mg/kg-bw per day) plus 3-day intraperitoneal injection of 17β-estradiol, decreased uterophic effect of 17β-estradiol, increased glucuronidation and enhanced *in vivo* metabolism of 17β-estradiol (Zhu et al. 1997).
Genotoxicity and related endpoints: *in vivo*	**Unscheduled DNA synthesis** *Negative*: B6C3F1 mice, forestomach epithelium 48 h single dose study, 300 mg/kg-bw (Benford et al. 1994). **DNA adduct formation** *Negative*: F344 rat forestomach (Hirose et al. 1987; Saito et al. 1989; Ito et al. 1991); stomach (Ito et al. 1991). **DNA strand breaks and repair** *Positive*: ddY mouse, stomach, colon (Sasaki et al. 2002). *Negative*: F344 rat hepatocytes (Williams et al. 1990a); F344 rat forestomach epithelium (Morimoto et al. 1991). **Sex-linked recessive lethal** *Negative: Drosophila* (Miyagi and Goodheart 1976). *Positive: Drosophila* (weak) (Prasad and Kamra 1974).
Genotoxicity and related endpoints: *in vitro*	**Reverse mutation** *Negative*: *Salmonella typhimurium* TA98, TA100, TA102, TA104, TA1535, TA1537 and TA1538, *Saccharomyces cerevisiae* strain D7; with and without activation (Joner 1977; Bonin and Baker 1980; Kawachi et al. 1980; Hageman et al. 1988; Matsuoka et al. 1990; Williams et al. 1990a, Rogers et al. 1992). **Gene mutation** *Positive*: *Staphylococcus aureus* without activation (Degre and Saheb 1982). *Negative*: HGPRT locus in ARL cells without activation (Williams et al. 1990a); Chinese hamster V79 cells with or without activation (Rogers et al. 1985). **Chromosomal aberrations** *Positive*: Chinese hamster ovary (CHO) with activation (Phillips et al. 1989; Matsuoka et al. 1990; Murli and Brusick 1992). *Negative*: Hamster, Don, Chinese hamster lung, CHO cells without activation (Abe and Sasaki 1977; Ishidate and Odashima 1977; Phillips et al. 1989; Matsuoka et al. 1990; Murli and Brusick 1992).

Endpoint	Lowest effect levels[1]/Results
	Sister chromatid exchange *Positive*: Hamster, Don cells without activation (Abe and Sasaki 1977). *Negative*: Hamster CHO cells with and without activation (Williams et al. 1990a), V79 (Rogers et al. 1985). **Unscheduled DNA synthesis** *Negative*: Rat, hepatocyte (Williams et al. 1990a).
Humans	
Short-term	BHA 0.5 mg/kg-bw ingested daily for 10 days, 8 male non-smoking volunteers, assessment of standard clinical plasma parameters (L-aspartate aminotransferase, L-alanine aminotransferase, L-gamma-glutamyltranspeptidase, creatine phosphokinase, lactate dehydrogenase, total protein, albumin, urea, creatinine, Na^+ and Cl^-), phase I and phase II biotransformation capacity and saliva/urine kinetic parameters. No significant differences were detected in the parameters measured; urinary excretion of metabolites of BHA was significantly increased on days 3 and 7 vs. the first day of BHA administration (Verhagen et al. 1989).
Chronic toxicity/ carcinogenicity (more than 3 months)	Case–cohort study assessing dietary intake of BHA and stomach cancer. Mean intake was 105 µg/day (range 2–3220 µg/day). No association of BHA intake with risk of stomach cancer was observed (Botterweck et al. 2000).
Miscellaneous studies	0.5–0.7 mg/kg-bw, 22–70% excreted in urine as glucuronide within 24 h. Less than 1% was excreted in the urine as unchanged BHA, and no dealkylation of hydroxylation products was detected (Astill et al. 1962). 0.5 mg/kg-bw of ^{14}C-labelled, single oral dose BHA, 67% of the radioactivity was excreted in the urine after 2 days; by day 11 post-dosing, 80–86.5% of the radioactivity was recovered in the urine (Daniel et al. 1967). 1.81 or 1.67 mg/kg-bw, two healthy male adults, single oral dose. Blood samples were withdrawn at 0.5, 1, 2, 4, 6 and 8 h following administration, and urine samples were collected for 0–8, 8–16, 16–24 and 24–36 h intervals. Less than 1% of dose was eliminated in the urine in 36 h. In both subjects, peak urinary levels occurred in the period between 8 and 16 h following dosing (El-Rashidy and Niazi 1979).

[1] LD_{50} = median lethal dose; LOEL = lowest-observed-effect level; NOAEL = no-observed-adverse-effect level; NOEL = no-observed-effect level.