Amended Safety Assessment of BHA as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date **(i.e., until June 9, 2024**) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

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ABBREVIATIONS

2-BHA	2-*t*-butyl-4-hydroxyanisole
3-BHA	3-*t*-butyl-4-hydroxyanisole
ADI	acceptable daily intake
Akr1c14	aldo-keto reductase family 1 member C1
CAS	Chemical Abstracts Service
CD36	platelet glycoprotein 4
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
CSCP	California Safe Cosmetics Program
CTFA	Consumer, Toiletry, Fragrance Association
Cyp11a1	cholesterol side-chain cleavage enzyme P450scc
DHT	5-α-dihydrotestosterone
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
E_2	17β-estradiol
EC_{50}	half maximal effective concentration
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
eIf2α	eukaryotic initiation factor 2
ELISA	enzyme linked immunosorbent assay
EPP	ethylphenyl proprionate
FCA	Freund's complete adjuvant
FDA	Food and Drug Administration
GADD153	growth arrest and DNA damage inducible gene 153
GRAS	generally recognized as safe
GRP78	glucose-regulated protein 78
GSH	glutathione
HRIPT	human repeated insult patch test
Hsd3b1	3β-hydroxysteroid dehydrogenase/λ(4) isomerase type 1
IL-6	interleukin-6
IRE1α	inositol-requiring enzyme-1
LD	lethal dose
Lhcgr	luteinizing hormone/chorionic gonadotropin receptor
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
MDSS	maximal primary Draize irritation score
MMAD	mass mean aerodynamic diameter
MNNG	*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine
MOS	margin of safety
MSP23	peroxiredoxin I
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
mRNA	messenger ribonucleic acid
MW	molecular weight
NACDG	North American Contact Dermatitis Group
NK	natural killer
NOAEL	no-observed-adverse-effect-level
NoG	Notes of Guidance
OECD	Organisation for Economic Co-operation and Development
PAH	*p*-aminohippurate
Panel	Expert Panel for Cosmetic Ingredient Safety
PII	primary irritation index
PPARγ	peroxisome proliferator-activated receptor gamma
REACH	Registration, Evaluation, Authorization, and Restriction of Chemicals
RNA	ribonucleic acid
Scarb1	scavenger receptor class B type 1
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose

SLS	sodium lauryl sulfate
Srd5a1	3-oxo-5α-steroid-4-dehydrogenase
Srebp1c	sterol regulatory element-binding protein 1
Star	steroidogenic acute regulatory protein
TG	test guideline
TNF-α	tumor necrosis factor-alpha
US	United States
UV	ultraviolet radiation
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of BHA, which is reported to function as an antioxidant and a fragrance ingredient in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that BHA is safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of BHA as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), this ingredient is reported to function in cosmetics as an antioxidant and a fragrance ingredient.[1]

The Panel first published a review of the safety of Butylated Hydroxyanisole (since renamed as BHA) in 1984.[2] Based on the available data presented in the report, the Panel concluded that BHA is safe as a cosmetic ingredient in the present practices of use (as described in the safety assessment). The Panel also previously considered a re-review of this report in September 2003 and reaffirmed the 1984 conclusion, as published in 2006.[3]

In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment was issued. At its June 2023 meeting, the Panel determined that this safety assessment should be reopened to evaluate potential endocrine and reproductive effects of BHA at high doses and to provide an updated assessment of the ingredient.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted February 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

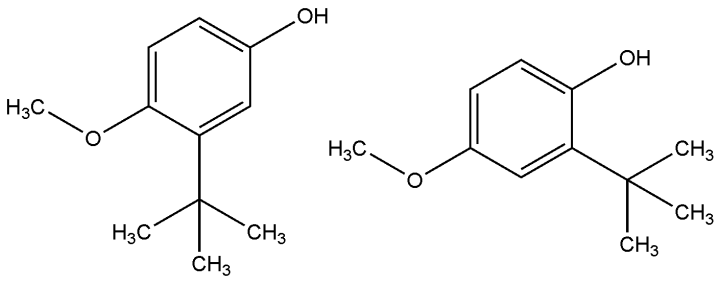
Summarized excerpts from the initial report on BHA and an unpublished document containing data considered by the Panel during the initial re-review process in September 2003 are included in this document, as indicated by *italicized* text. (This information is not included in the tables or the summary section.)

Of note, the *Dictionary* defines BHA as a mixture of tert-butylated 4-hydroxyanisole isomers which consists chiefly of 3-*tert*-butyl-4-hydroxyanisole with lesser amounts of 2-*tert*-butyl-4-hydroxyanisole. Thus, data found on BHA in both isomeric forms has been included and identified when available; in cases where the isomeric form is not known, the test article is simply described as BHA. Some toxicological data on BHA included in this safety assessment were obtained from robust summaries of data submitted to the European Chemicals Agency (ECHA) by companies as part of the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) chemical registration process.

CHEMISTRY

Definition and Structure

According to the *Dictionary*, BHA (CAS No. 25013-16-5) is mixture of tertiary butyl-substituted 4-methoxyphenols.[1] It consists chiefly of 3-t-butyl-4-hydroxyanisole (3-BHA) with lesser amounts of 2-t-butyl-4-hydroxyanisole (2-BHA), with structures as shown in Figure 1.



3-(*tert*-butyl)-4-methoxyphenol 2-(*tert*-butyl)-4-methoxyphenol

Figure 1. BHA

Chemical Properties

BHA (MW = 180.2 g/mol) is a white or slightly yellow, waxy solid having an aromatic odor.[2] BHA exhibits antioxidant properties and acts synergistically with acids, butylated hydroxytoluene, propyl gallate, hydroquinone, methionine, lecithin, and thiodipropionic acid in protecting lipids autooxidation. The use of a synergistic combination will result in a greater

stability than can be obtained by using the equivalent quality of either antioxidant alone. A BHA sample of > 99% purity has a protein-binding capacity of 4680 mmol/mole protein and a hydrophobic bonding ability (expressed as the difference in log partition coefficients of BHA and phenol) of 1.88. BHA also has a log K_{ow} of 3.5 and a water solubility of 0.213 g/l at 25 °C.[4] Other chemical properties of BHA can be found in Table 1.

Method of Manufacture

BHA can be synthesized either by tert-butylation of p-methoxyphenol or by methylation of t-butylhydroquinone.[2] BHA can also be prepared from p-methoxyphenol and isobutene. The product is purified by distillation and subsequently supplied to cosmetic formulators in the form of tablets or flakes.

Impurities

The following impurities have been reported for BHA as it is used in cosmetics: 4-hydroxyanisole (0.5% maximum), 1-t-butyl-2,5-dimethyoxybenzene (0.5% maximum), 2,5-di-t-butyl-hydroxyanisole (0.2% maximum), hydroquinone dimethyl ether (0.1% maximum), sulfated ash (0.01% maximum), lead (as Pb; 20 ppm maximum), and arsenic (as As; 3 ppm maximum).[2] Food-grade BHA is also reported to contain hydroxyanisole and hydroquinone at levels of 0.5 and 0.6% (maximum) respectively.

Specifications for food-grade BHA include an acceptance criteria of no-less-than 98.5% BHA in a given sample.[5]

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics and does not cover its use in airbrush delivery systems. Data included herein were obtained from the FDA's Voluntary Cosmetic Registration Program (VCRP) database in 2023 (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data were provided by cosmetic product categories, based at that time on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, BHA is reported to be used in 70 formulations (Table 2).[6] At the time this ingredient was last considered for re-review, 1224 uses were reported.[3] The results of the concentration of use survey conducted by the Council in 2023 indicate that the maximum reported concentration of use for BHA is at 0.15% in other manicuring preparations; the highest concentration of use reported for a leave-on dermal exposure is at up to 0.05% in face powders.[7] BHA was reported to be used at 0.2% in several product formulations (cologne and toilet waters, perfumes, blushers, and lipstick) in 2003.[3] Overall, the reported frequency of use for BHA has decreased significantly while the reported concentrations of use have remained constant with no new use categories.

BHA is reported to be used in products used near the eye; for example, it is used at up to 0.05% in eyeliner and eyeshadow and at 0.03% in mascara. BHA is reported to be used at 0.05% in lipstick, a product that can be incidentally ingested. BHA is also reported to be used in cosmetic sprays (e.g., other fragrance preparations at 0.001%), in spray deodorants (at 0.000051%), and in face powders at 0.05%, and could possibly be inhaled. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable. However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

Although products containing this ingredient may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of this ingredient (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

BHA is not restricted from use in any way under the rules governing cosmetic products in the European Union.[8]

Non-Cosmetic

Studies have suggested that BHA is mainly detected in various foods, including fats, oil, and their products; cereals and cereal products; vegetables and vegetable products; milk and milk products; meat and meat products, and in fish, fish products, and seafood.[9] According to 21CFR582.3169, BHA is generally recognized as safe (GRAS) for use in foods when the total content of antioxidant is not over 0.02% of fat or oil content, including essential (volatile) oil content of the food, provided the substance is used in accordance with good manufacturing or feeding practice. BHA is also approved for various uses in food contact and food packaging materials, including: as a pressure-sensitive adhesive (21CFR175.125), as an antioxidant in resinous and polymeric coatings (21CFR175.300), as a defoaming agent (21CFR176.210), and as a component of polyethylene film (21CFR179.45).

In 2019, the European Food Safety Authority (EFSA) determined that the maximum authorized BHA content in animal feed (all animals, except cats), either alone, or in combination with butylated hydroxytoluene and/or ethoxyquin is 150 mg/kg.[10] In 2011, based on a no-observed-adverse-effect-level (NOAEL) of 100 mg/kg bw/d BHA for growth retardation, increased mortality and behavioral effects in rat pups at higher dose levels, and using an uncertainty factor of 100, the EFSA Panel on Food Additives and Nutrient Sources added to Food revised the acceptable daily intake (ADI) of BHA from 0.5 mg/kg bw/d to 1 mg/kg bw/d.[11]

TOXICOKINETIC STUDIES

Dermal Absorption

In Vitro

The dermal absorption of BHA was measured in an in vitro preparation of human skin.[12] After a 16-h continuous application of 0.07% BHA, approximately 30% of the applied amount remained on the skin surface, approximately 6% was found in the horny layer, 50% penetrated to the dermis and epidermis, and 2.68% of the applied BHA penetrated to the receptor fluid.

Absorption, Distribution, Metabolism, and Excretion

Animal

Oral

Multiple studies in rats have established that BHA is absorbed from the gastrointestinal tract and metabolized.[2] Groups of 4 or 8 male and female Sprague-Dawley rats were administered a single oral dose of BHA at up to 400 mg/kg, in various isomeric forms. Urinary excretion of BHA glucuronide and ethereal sulfate during 5 d post-dosing accounted for 61 – 82 and 11 – 16% of the recovered dose, respectively; 5% of the dose was excreted unchanged. For single oral doses of 2, 10, 25, 50, 100 mg/kg BHA overall recovery in the urine was 81 – 100%, with a slight increase in the excretion of unchanged BHA at the lower doses. A single oral dose of 400 mg/kg 2-BHA was excreted in 5 d; 72% of the dose was excreted as ethereal sulfate. For 3-BHA, 57 – 71% of a 400 mg/kg dose was excreted as glucuronide in 5 d. Several successive daily doses of 500 mg/kg BHA and of 3-BHA decreased the percent recovery of conjugates and the proportion of excreted ethereal sulfate. Repeated doses of 500 mg/kg 2-BHA resulted in a considerable increase in glucuronide and a decrease in ethereal sulfate conjugation. The researchers suggested that 4-O-conjugation, O-demethylation, hydroxylation of the phenyl ring, and sidechain oxidation (-CH_2, -CH_2OH) may be involved in the metabolism of BHA. The possibility of O-demethylation of the 2-BHA isomer as an alternative metabolic pathway was also recognized. Ninety percent of a single dose of 80 – 100 mg BHA given orally to rats was excreted in the urine as glucuronide conjugate (71%), ethereal sulfate (14%), and unconjugated phenol (5%).

In the rabbit, 2-BHA and 3-BHA are metabolized largely by 4-O-glucuronide formation. Rabbits given 1000 mg BHA in olive oil via gavage (approximately 500 mg/kg) excreted 46% of the dose as glucuronides, 9% as ethereal sulfates, and 6% as free phenols. The corresponding recovery rates after an oral dose of 125 mg/kg BHA were 84, 18, and 19%, and were 60, 12, and 4% after a 500 mg/kg BHA dose, respectively.

In dogs, BHA is absorbed in the intestine only to a small extent and is excreted after being combined with sulfuric acid. Three dogs were administered 350 mg/kg BHA in lard, in the diet. Sixty percent of the antioxidant was excreted unchanged in the feces within 3 d. The remainder of the dose was excreted in the urine as ethereal sulfate (23%), t-butyl hydroquinone (5.5%), free BHA (3.6%), and as an unidentified phenol. Fasted dogs given dietary doses of 50 and 250 mg/kg BHA in the diet had ethereal sulfate and glucuronides in the urine.

The Select Committee on GRAS Substances has suggested that tissue storage of BHA may occur because of the lipid solubility of the antioxidant. However, they noted that the amount stored may be quite limited due to rapid metabolism and excretion. Concordantly, only trace amounts of BHA were found in the depot and carcass fat of rats given 2-3% BHA in the diet for 6 mo. A disposition half-life of approximately 1 h was estimated for a single intravenous dose of 10 mg/kg in rabbits. Pigs that were fed 0.1% BHA in the diet for 4 mo and pullets that were fed 0.1% BHA in the diet for 8 wk showed no accumulation of BHA in the muscle, liver, kidney, or the reserve fat. No storage of BHA in the body fat, brain, liver, or kidney occurred in groups of dogs that consumed a 1- yr diet containing up to 100 mg/kg BHA as a 50% solution in propylene glycol.

Rats were administered a single, 1000 mg/kg bw oral dose of ^{14}C- labelled BHA.[12] Within 2 d of dosing, 87 – 96% of the ^{14}C was excreted, mainly in the urine with smaller amounts in the feces and expired air. More ^{14}C was found in the tissues of rats given the methoxy-labelled compounds. The distributions of ^{14}C in the forestomach and the glandular stomach were similar. After 168 h of treatment, more ^{14}C was found in the forestomach of rats given 2-BHA than in that of rats given the 3-BHA isomer. The researchers considered these results to indicate that the excretion of BHA is rapid, that 4-O-methyl demethylation may take place readily, and that the demethylated methyl group may become distributed non-specifically in tissues.

Rats were fed 0.12% 3-BHA in the diet for 21 mo.[13] The test substance was absorbed from the gut via passive diffusion. No evidence of tissue storage was observed in the animals. No further details were provided.

Human

Oral

The majority of a single, 100 mg oral dose of BHA administered in a gelatin capsule to an unspecified number of human subjects was eliminated in the urine as glucuronides (~44%), sulfates (~26%), and O-demethylated metabolites (~42%); less than 1% was recovered as the intact compound.[2] In a second study, a single dose of 40 mg ^{14}C-labelled BHA was orally administered to 2 men. In 2 d, 60 – 73% of the radioactivity was excreted in the urine, and within 11 d, 80 – 87% appeared in the urine. It was suggested that the delay in excretion may be due to prolonged enterohepatic circulation or to a slow release of the compound and its metabolites from tissue storage. In a third study, 6 adult men received a single, 0.4 – 0.7 mg/kg oral dose of BHA either in a capsule (50 mg) or oil-milk emulsion (31 mg). Twenty-seven to 77% of the dose was excreted in the urine as glucuronide within 23 – 38 h. Less than 1% of the administered doses appeared in the urine as ethereal sulfates or as free BHA, and no dealkylation or hydroxylation products were detected. The time required for excretion of the administered dose varied from 23 – 50 h.

The absorption and excretion of BHA has been evaluated in human subjects.[12] Four subjects were given a single oral dose of 5 or 30 mg BHA in olive oil. Plasma concentration of BHA peaked at 73.03 ng/ml (108.75 mins after the 30 mg dose) and at 14.14 ng/ml (142 min after the 5 mg dose. The half-life of BHA in plasma was 2.79 h for the 5 mg dose and 2.96 h for 30 mg dose. About 20% of the administered dose was excreted as BHA-glucuronide in the urine within 24 h. Only 0.03% of the administered dose was excreted as free BHA.

Subjects (sex and number not specified) received a single, oral dose of 0.5 mg/kg 3-BHA.[13] Most of the test substance was recovered in the urine and feces (95%). 3-BHA was excreted in its conjugated form in the urine and as conjugated *t*-butyl hydroquinone in the feces. No free 3-BHA was found in the urine or feces.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

The acute dermal toxicity of an eye makeup preparation containing 0.1% BHA was evaluated using rabbits (number unspecified), in accordance to guidelines outlined in 16 CFR 1500.40.[2] The dermal LD_{50} was determined to be > 2000 mg/kg.

Oral

The acute oral LD_{50} values of BHA administered in olive oil to male and female mice were 1100 mg/kg and 1320 mg/kg, respectively.[2] The acute oral LD_{50} values for BHA in 2 additional studies using mice were 1250 and 2000 mg/kg. The acute oral LD_{50} values for male rats were determined to be 2800 mg/kg (BHA in isopropyl alcohol) and 2950 mg/kg (BHA); the acute oral LD_{50} values for BHA in rats were determined to be 2200 mg/kg and 2900 mg/kg in 2 separate studies. For male and female rats, the acute oral LD_{50} for BHA administered in olive oil was determined to be 2000 mg/kg. Non-fasted rats received a single oral dose of BHA in corn oil; the acute oral LD_{50} was 4100 mg/kg. Fasted rats were administered a single oral dose of BHA in water; the acute oral LD_{50} was > 5000 mg/kg.

Cosmetic products containing BHA have also been evaluated for their potential to cause acute oral toxicity. In 2 separate studies, a single oral dose (5000 mg/kg) of a suntan preparation containing 0.1% BHA or an eye shadow containing 0.2% BHA were administered, via gavage, to female albino rats (n=5). The acute oral LD_{50} for both products was > 5000 mg/kg. The acute oral LD_{50} of an eye makeup preparation containing 0.1% BHA was also > 5000 mg/kg in rats.

The acute oral LD_{50} of BHA, administered in dimethyl sulfoxide (DMSO) to mice, was 1670 mg/kg; the acute oral LD_{50} of BHA, administered in olive oil to mice was 1583 mg/kg.[12] A single oral dose of BHA was administered to rats, in DMSO or in olive oil; the corresponding acute oral LD_{50} values were 2910 mg/kg and 2960 mg/kg, respectively.

Short-Term Toxicity Studies

Oral

Four male Sprague-Dawley rats were given 500 mg/kg/d BHA via gavage for 6 d.[2] Total daily sodium excretion in the urine of treated animals was less than expected from food intake on days 2 -6, possibly owing to interference with renal prostaglandin synthesis. Male rats were given 500 mg/kg/d BHA in the diet for 1, 2, 4, or 6 d; p-aminohippurate (PAH)

accumulation was reduced in the kidney after 1 dose of BHA. Increases in liver weight following the second dose were accompanied by increases in PAH serum to slice ratio, but these increases approached normal levels after 6 doses. Male and female SPF Carworth rats (n = 4 – 12/sex/group) were given 0, 50, 100, 200, or 500 mg/kg/d BHA for 7 d, via gavage. No changes in liver fat were noted; however, significant increases in liver weights were observed in females in the 50, 200, and 500 mg/kg/d groups and in males from the 100, 200, and 500 mg/kg/d groups. No changes were observed with respect to fat metabolism, weight gain, liver glycogen, cholesterol, phospholipids, or concentration and iodine number of liver fat, in rats that were administered 4 mg/kg/d BHA in the diet for 30 to 35 d. Male and female Norway hooded rats (6/sex/group) received 0, 0.1, 0.2, 0.3, 0.4, or 0.5% BHA) in the diet for 6 wk. Increased levels of total serum cholesterol were noted in animals fed 0.1% BHA. Increasing dietary concentrations of BHA were associated with increased absolute lipid content and liver weight; no histologic changes were attributable to treatment. No changes were observed with respect to growth, composition of hepatic polyunsaturated fatty acids, serum levels of sodium, total liver lipid concentration, or total and esterified cholesterol levels of liver and adrenals. Eight rats were given 400 mg/kg/d BHA in the diet for 8 wk; besides a decrease in phagocytic activity of leukocytes, no change in various blood characteristics was observed. Rats consumed 500 mg/kg/d BHA for 82 d or 600 mg/kg/d BHA for 68 d, both in the diet. Lag in weight gain and reduced blood catalase and peroxidase activities and increases in liver weights and body fat content were observed; however, upon necropsy, no pathologic differences were noted between treated and control animals. Female Carworth Farm SPF rats (n = 4 - 5) were administered 500 mg/kg/d BHA for 84 d, via gavage. Significant increases in liver weights and liver protein were observed; activities of hepatic hexobarbital oxidase, nitroanisole demethylase, codeine demethylase, and aminopyrine demethylase were not affected. Researchers thought it appropriate to disregard hyperfunctional liver enlargement in assessing the acceptability of BHA as a food ingredient. Male and female weanling Carworth SPF rats (n = 24/sex) were administered a diet containing 0.1% BHA (for up to 16 wk. Toward the end of the 16-wk period, there was a decrease in food consumption and retardation of growth in males. Increases in relative liver weight, and in a few instances, adrenal weights, occurred predominantly in females. No significant changes in hepatic glucose-6-phosphatase occurred in either sex after 16 wk; however, a decrease in this enzyme's activity was noted in females after 4 wk. No histopathologic evidence of damage to the liver was observed.

A 1000 mg/kg dose of BHA, administered in olive oil via gavage for 1 to 7 d, was established to be lethal in rabbits. The researchers suggested that the primary effect of BHA may have been renal, resulting in excessive loss of salt (potassium and sodium) in urine, and within muscle, and other, tissue.

Nine infant and 17 juvenile rhesus monkeys of both sexes were given 500 mg/kg BHA, and 17 juvenile rhesus monkeys of both sexes were given 50 mg/kg BHA for 28 d, via gavage. A pronounced increase in relative liver weights was observed in juvenile monkeys given 500 mg/kg/d; monkeys given 50 mg/kg/d BHA showed enlarged livers, which was of questionable significance. Histological evaluation of the liver of juvenile monkeys revealed cytomegaly and enlargement of cell nucleoli; no other treatment-related changes were seen in the organs of infants or juveniles. Similarly, adolescent rhesus monkeys were given 500 mg/kg/d BHA for 4 wk, via gavage; no significant changes were seen in weekly blood counts, electrolyte determinations, liver function tests, microsomal levels of ribonucleic acid (RNA), phospholipids, or cytochrome P450. Accumulation of liver lipids was 25% above untreated controls. Electron microscopy revealed marked proliferation of smooth endoplasmic reticulum and enlarged nuclei and nucleoli; hepatic nucleoli were fragmented and contained a dense network of coarse fibrils. Monkeys were given 0 (n = 2), 50 (n = 3), or 500 mg/kg (n = 4) BHA for 28 d in the diet; total cholesterol levels in the plasma and liver significantly lowered at the 500 mg/kg/d dose; treatment at 50 mg/kg/d significantly lowered liver cholesterol. The researchers suspected a relationship between large doses of BHA, the level of dietary vitamin E, and the type and level of dietary lipid with respect to their role in primate lipid metabolism.

BHA, at 0.7% mixed in the feed of Syrian golden hamsters for 2 wk, induced superoxide dismutase in the liver but not in the brain.[12] BHA reduced DT-diaphorase activity in the brain by 40%. Glutathione (GSH)-related enzyme activities in the brain were not affected by BHA, but BHA increased GSH S-transferase and GSH-reductase in the liver. These results indicated that the permeability of the blood-brain barrier to BHA is limited.

BHA administered, via gavage, to female cynomolgus monkeys 5 d/wk for 84 d produced transient changes in selected serum chemistry and hematology parameters. Terminal observations revealed increased liver size, decreased hepatic monooxygenase activity and an increase in the mitotic index of the esophageal epithelium. Gastroscopic evaluation of the stomach and esophagus at monthly intervals and extensive gross and histopathological examination failed to reveal proliferative effects seen in the forestomach of rats fed diets containing BHA.

The lowest-observed-effect-level (LOEL) values for rats dosed "continuously" with 3-BHA for 6 wk, via gavage, was determined to be 63,000 mg/kg bw.[13] No further details were provided.

Subchronic Toxicity Studies

Oral

Rats (n = 20/group) were given 0. 0.05, 0.15, 0.45, or 1.35% BHA in the diet for 110 d.[2] Males in each treatment group exhibited increased liver weight but normal weights for brain, pituitary, thyroid, thymus, heart, testis, prostate, spleen, and adrenals; male rats fed 0.05% BHA showed increased lung and kidney weights. Female animals demonstrated increased thymus weight at 0.05%, increased thyroid weight at 0.15, 0.45, and 1.35%, and increased liver weight at all dose levels.

Brain, pituitary, heart, lung, spleen, adrenal, kidney, uterus, and ovary weights of female rats were comparable in all treated female groups, compared to controls. Microscopic examination of the kidneys of several animals of both sexes revealed necrosis (all dose levels), expansion of the renal cavity (all dose levels), and epithelial swelling in the tubules (0.15, 0.45, and 1.35%).

The LOEL for rats dosed "continuously" with 3-BHA, via gavage, for 16 wk was determined to be 9900 mg/kg bw.[13] No further details were provided.

Two groups of male C57BL/6J mice (24/group) were fed a normal diet (10 kcal% from fat) or a high-fat diet (45 kcal% from fat).[14] Both natural diet and high-fat diet groups were randomly categorized into 3 groups (8/group) to receive either 0.2% DMSO or 1 or 10 mg/kg bw 3-BHA via gavage for 18 wk. (Doses were based on the human ADI of 1 mg/kg bw/d). Mice in the natural diet group steadily gained body weight at approximately 0.48 g/wk, which was irrespective of BHA administration. Animals in the 1 mg/kg 3-BHA, high-fat diet group had significantly decreased body weight gain at 0.49 g/wk, while rats in the 10 mg/kg group had slightly increased body weight gain at 0.68 g/wk. Patterns of inguinal subcutaneous white adipose tissue and perigonadal visceral white adipose tissue accumulation were consistent with body weight gain in BHA-treated, high-fat diet mice. Upon BHA treatment, the mRNA levels of adipogenic transcriptional factors, *PPAR*γ (peroxisome proliferator-activated receptor gamma), *Srebp1c* (sterol regulatory element-binding protein 1), CD36 (platelet glycoprotein 4), *IL*-6 (interleukin-6), and *TNF*- α (tumor necrosis factor alpha) in perigonadal visceral white adipose tissue were significantly increased in a dose-dependent manner for both natural and high-fat diet groups ($p < 0.05$ or 0.01) Glucose metabolism and insulin sensitivity remained unaffected.

Chronic Toxicity Studies

Oral

Albino rats and guinea pigs were given 4 mg/kg/d BHA, in the diet for 6 mo.[2] Rats showed transient eosinopenia beginning in the fifth month. Guinea pigs showed a temporary drop in urinary 17-oxycorticosteroids after 4 mo. Wistar albino rats of both sexes were give BHA in the diet (1.35 or 67.5 mg/kg) for 32 or 52 wk. No deleterious effects with respect to survival, growth, organ weights, hemoglobin levels, or histopathologic changes in organs were treatment-related. Hooded Norway rats (n = 80) were fed up to 0.5% BHA in the diet for 8 mo and albino rats (n = 26) were fed up to 0.1% BHA in the diet for 2 yr. A reduction in mature weight and an increase in relative liver weight was noted at the 0.5% BHA dose. No effect was seen at any level in hooded Norway or albino rats with respect to mortality, reproductive cycle, histology of spleen, testes, kidney, liver, skin, or relative weights of spleen, heart, and kidney. Six rats were administered 2% BHA in the diet for 6 mo and 68 weanling rats were administered 0.12% BHA in the diet for 21 mo. Weight gains were decreased in rats fed 2% BHA for 6 mo; histopathological examination revealed no adverse effects attributable to BHA treatment. No significant differences were seen in weight gain, growth, reproduction, or histopathology in the weanling rats fed 0.12% BHA for 21 mo, compared to controls.

Four weanling cocker spaniel dogs received 0, 5, 50, or 250 mg/kg/d BHA in the diet for 15 mo. No appreciable gross, hematological, or microscopic changes in organs (with the exception of the liver) were noted between treated animals and controls. Urine from dogs fed BHA contained higher levels of glucuronates and higher ratios of total to inorganic sulfates, compared to controls. Dogs fed 250 mg/kg/d gained less weight and consumed less food than controls. Three out of 4 dogs in the 250 mg/kg/d group showed liver parenchymal degeneration, as well as diffuse granulocytic infiltration accompanied by marked narrowing of hepatic sinusoids. It was noted that these 3 dogs each consumed more than 1500 mg/kg/d BHA during the 15-mo period, compared to the fourth dog which ate only half of the daily ration (786 mg BHA/d or 183 mg/kg BHA). Groups of beagle dogs (3/group) were given up to 100 mg/kg/d BHA in the diet as a 50% solution for 1 yr, or orally at 30 mg/kg/d in a solution containing 20% BHA (and 6% propyl gallate, 4% citric acid, and 70% propylene glycol) for 1 yr. Blood sample values and organ weights were within normal ranges. No storage of BHA in fat, brain, liver, or kidney, and no increase in urinary reducing substances was observed. Histologic examination of heart, lungs, spleen, stomach, small and large intestine, pancreas, liver, adrenals, kidneys, urinary bladder, thyroid, bone marrow, and brain revealed no evidence of changes attributable to BHA treatment.

Rats received 0, 0.125, 0.25, 0.5, 1, or 2% BHA, in the diet for 104 wk.[15] Body weights were reduced in rats receiving at least 0.5% BHA. Significant pathology was only seen in the forestomach epithelium, in animals exposed to > 0.5% BHA. No further details were provided.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

No discernible effect on nidation or on maternal or fetal survival were observed in female CD-1 mice that received up to 225 mg/kg/d BHA, via gavage, from day 6 to 15 of gestation.[2] The number of abnormalities seen in either soft or skeletal tissues of the test groups was similar to that occurring spontaneously in sham-treated controls. BHA was administered via oral intubation, in arachis oil, to ICI SPF female mice (500 mg/kg/d for 7 wk before mating and until day 18 of gestation; abnormalities were considered spontaneous, since they were also observed in untreated groups. Concurrently, BHA was orally administered to Tuck albino rats (750 mg/kg/d from day 1 to 20 of gestation), Tuck albino and Benger hooded rats (750 mg/kg/d for 70 d before mating and throughout gestation), Tuck and Carworth SPF albino rats (single administration of

1000 mg/kg on day 9, 11, or 13 of gestation), and Porton albino rats (500 mg/kg/d for 7 wk before mating and throughout gestation). All doses retarded growth of weanling albino female rats and produced weight loss in adults. However, no significant embryotoxic or teratogenic effects were seen in any strain of either species (albino or hooded). The abnormalities were considered spontaneous, since they were also observed in untreated groups. Negative teratogenic results were also reported for Wistar albino rats that received up to 200 mg/kg/d BHA from day 6 through 15 of gestation and for hamsters that received 120 mg/kg/d BHA from day 6 through 10 of gestation. No deleterious effects on reproduction in terms of 21-d litter weights or numbers of pups born and weaned were observed in 80 Norway hooded rats fed up to 0.5% BHA in the diet for 8 mo. Neither rats nor guinea pigs, that received 4 mg/kg/d BHA in the diet for 6 mo, showed impaired function of the reproductive glands. There was also no observed adverse effects in either animal with respect to sex cycle phases, activity of gonadotropic pituitary hormone glands, or histology of endocrine glands.

BHA was administered, via gavage, to pregnant SPF New Zealand rabbits at doses of 0, 50, 200, or 400 mg/kg/d from day 7 to 18 of gestation; fetuses were removed on day 28. No effect related to BHA treatment was observed on the number of corpora lutea, implantations, fetuses (dead or alive), or on gross malformations, skeletal and internal malformations, or on the weight of the fetuses. Intragastric administration of BHA to rabbits at doses up to 200 mg/kg on days 6 through 18 of gestation had an adverse effect on the survival of both dams and fetuses. This adverse effect did not appear to be dose-dependent. The ratio of resorptions to number of implant sites per dam was increased over the sham-treated controls in all dosage groups. However, the number of abnormalities observed in either skeletal or soft tissues of fetuses from test groups did not differ significantly from those occurring spontaneously in sham-treated controls. The researchers concluded, therefore, that BHA, while exhibiting systemic toxicity to the rabbit at the tested dose, was not a teratogen.

The embryotoxicity of BHA was evaluated in Danish Landrace SPF pigs.[2,12] Pregnant pigs were fed 0, 50, 200, or 400 mg/kg/d BHA from the time of artificial insemination to day 110 of gestation. The number of pregnant pigs in each treatment group totaled 9, 11, 13, 10, respectively. Fetuses were removed on gestation day 110 and examined for visceral and skeletal defects. Food consumption and appearance of dams were comparable between control and treatment groups. However, a significantly lower weight gain was observed in dams fed 400 mg/kg/d BHA. Necropsy of dams revealed only sporadic and common pathological lesions but no changes in reproductive organs. Hemoglobin, packed cell volume, total erythrocyte count, reticulocyte count, and differential leukocyte count were within normal range in all exposed groups. Absolute and relative weights of liver and thyroid showed a dose-related increase in the treated animals, but no histopathologic changes were noted in the liver. In the thyroid gland, large follicles with flattened epithelium containing thyroglobulin were seen in some animals, particularly in the high-dose group; the researchers suggested that the histologic changes in the thyroid gland indicated a reduced thyroidal activity. Major visceral and skeletal defects in fetuses were within the normal range. BHA did not affect reproduction as measured by pregnancy rate, number of implantations, number of corpora lutea, and did not show any significant teratogenic effects. Proliferative and parakeratotic proliferative changes of the stratified epithelium of the stomach were found in both control and treated pigs. In addition, proliferative and parakeratotic changes of the esophageal epithelium were observed in a few pigs in the 200 and 400 mg/kg groups. Papillomas were not found, and no changes of the glandular part of the stomach were observed.

Adult rhesus female monkeys (n = 6) were given 50 mg/kg/d BHA in the diet for 2 yr.[2] No abnormalities with respect to blood chemistry, menstrual cycles, food consumption, or body weight were observed during the first year in treated animals, compared to controls. Following the first year of exposure, treated females were bred to rhesus males that had received unmodified diets. Gestation was free of complications and treated animals delivered normal infants; hematologic evaluations of infants exposed during gestation and for 60 d after were similar to those of control infants. Infants and adults were observed for 2 yr following BHA exposure; adults continued to have normal infants and infants born during the exposure period remained healthy.

Details of the developmental and reproductive toxicity studies summarized below are found in Table 3.

The effect of BHA upon embryoid body development was evaluated in P19C5 stem cells.[16] The lowest-observed-adverse-effect-level (LOAEL) for BHA affecting embryoid body morphogenesis was 400 μM; BHA influenced the expression of developmental genes in a temporal and gene-specific manner. Groups of male and female Sprague-Dawley rats (12/sex/group) were given 0, 10, 100, or 500 mg/kg BHA, in corn oil, via gavage, in a generational developmental and reproductive toxicity study.[17] Weights of liver, adrenal and thyroid glands were increased, mating rate was decreased, and cohabitation during conception was longer in the F_0 (parental) generation. Body weights were significantly reduced in the 500 mg/kg group at postnatal day 21, liver and adrenal gland weights were increased, while weights of the spleen, vagina, testes, and ventral prostate were decreased in the F_1 (filial) generation rats exposed to 100 or 500 mg/kg BHA for 13 wk. Additionally, reduced velocity of sperm motion and number, lowered serum levels of thyroxine and testosterone, slightly shortened estrous cycle length, and effects in the follicular epithelial cells of the thyroid were observed in F_1 rats exposed to 500 mg/kg BHA. In another reproductive toxicity study, male and female rats received 0, 110, 220, or 420 mg/kg/d BHA, in the diet; the NOAEL for maternal toxicity was determined to be 420 mg/kg/d for.[15] For offspring toxicity, a NOAEL of 220 mg/kg/d BHA and a LOAEL of 420 mg/kg/d BHA was established, based on the reduced weight of progeny during lactation and increasing peri-weaning mortality.

ENDOCRINE EFFECTS

In a study evaluating the in vitro effects of BHA on testicular cell function, immature mouse Leydig cells and Sertoli cells were grown in serum-free medium to 60% confluence prior to treatment with 0, 10, 20, 50, 75, or 100 μM BHA for 24 h.[18] Cytosolic and mitochondrial calcium ion concentration levels were disrupted and endoplasmic reticulum stress signaling pathways were induced in Leydig and Sertoli cells, relative to vehicle-treated controls. The relative mRNA expression of genes involved in the biosynthesis of steroids were significantly downregulated after treatment with BHA in both cell lines.

The steroidogenic effects of BHA were evaluated in immature rat Leydig cells.[19] Cells were isolated from 35-d old rats and cultured with 50 μM BHA for 3 h, in combination with 22R-OH-cholesterol, pregnenolene, progesterone, androstenedione, testosterone, or dihydrotestosterone, and the concentrations of 5α-androstenediol and testosterone were measured in the media. Real-time polymerase chain reaction (q-PCR) was used to measure the mRNA expression levels of the following genes: *Lhcgr* (luteinizing hormone/chorionic gonadotropin receptor), *Scarb1* (scavenger receptor class B type 1), *Star* (steroidogenic acute regulatory protein), *Cyp11a1* (cholesterol side-chain cleavage enzyme P450scc), *Hsd3b1* (3β-hydroxysteroid dehydrogenase/λ(4) isomerase type 1), *Srd5a1* (3-oxo-5α-steroid-4-dehydrogenase), and *Akr1c14* (aldo-keto reductase family 1 member C1). The testis microsomes were prepared to detect the direct action of BHA on HSD3B1, 17α-hydroxylase (CYP17A1), and 17β-hydroxysteroid dehydroxygenase-3 activities. BHA significantly inhibited androgen production, rat testis CYP17A1 and HSD3B1 activity, and expression levels of *Hsd17b3* and *Srd5a1*, leading to lower production of androgen in Leydig cells.

BHA was evaluated for its anti-androgenic activity in a reporter gene assay and an androgen receptor antagonist test using the human MDA-kb2 breast cancer cell line.[20] For the androgen receptor agonist test, cells were exposed simultaneously to a constant concentration of 1000 pM 5-α-dihydrotestosterone (DHT), a strong androgen receptor agonist, with increasing concentrations of each test material (0, 0.15, 0.5, 1.5, 5, 15, 30, 50 150, or 300 μM), in DMSO When tested in the presence of 1000 pM DHT, BHA exhibited significantly anti-androgenic activity in a concentration-dependent manner. At the highest concentration (300 μM), BHA completely inhibited DHT-induced luminescence

In another luciferase assay, the anti-androgenic effects of BHA was evaluated in transfected human PC-3 prostate carcinoma cells.[21] Cells were incubated for 18 h with each test material (0, 0.1, 1, or 10 μM) in the presence or absence of 50 pM DHT. BHA did not inhibit androgenic activity in the absence of DHT, but did antagonize DHT-induced activation of the androgen receptor such that at the 10 μM test concentration the DHT-induced effect was inhibited by at least 50%. Cell viability was not affected by treatment with BHA. The half maximal effective concentration (EC_{50}) for BHA to inhibit DHT-mediated activation in the luciferase assay was 7.6 μM; BHA was considered a partial androgen antagonist.

The estrogenic and anti-estrogenic effects of BHA were evaluated in an luciferase assay using T47D-Kbluc breast cancer cells and in an estrogen-dependent proliferation assay, using MCF-7 breast cancer cells.[22] In the luciferase assay, cells were treated with increasing concentrations (0, 0.15, 0.5, 1.5, 5, 15, 30, 50, 150, or 300 μM), in DMSO, for 24 h; estradiol was used as a positive control, at concentrations of 30 pM and 10 pM, in each assay. Anti-estrogenic effects were evaluated in the proliferation assay; cells were incubated in the presence of 10 pM estradiol. BHA exhibited a higher potency of estrogenic effects in the luciferase assay than in the proliferation assay.

The anti-androgen-, glucocorticoid-, and thyroid hormone-like activities of BHA were evaluated in multiple luciferase assays, using MDA-kb2 and GH3.TRE-Luc reporter cell lines.[23] BHA exhibited anti-glucocorticoid-like and anti-androgen activity

The androgenic activity of BHA was evaluated in a Hershberger assay.[24] Male Sprague-Dawley rats (42-d-old; 8/group) had the testis and epididymis removed. After 8 d of recovery, animals received 0, 50, 100, 250, or 500 mg/kg BHA in corn oil, via gavage, for 10 d. Groups of the tested animals received a single, subcutaneous injection of testosterone propionate, dissolved in corn oil, at a volume of 0.4 ml/kg. After final treatment, animals were weighed, and androgen-dependent accessory sex glands or organs, and liver, kidney, adrenal, and thyroid glands were removed and weighed. Body weight gain was significantly affected by testosterone propionate alone and combined with 250 mg/kg BHA. Relative liver and adrenal weights were significantly increased by treatment with 500 mg/kg BHA alone; relative adrenal gland weight was significantly decreased by testosterone propionate alone and in combination with 250 mg/kg BHA. The relative and absolute weights of the ventral prostate, seminal vesicle with coagulating glands, glans penis, *levator ani plus bulbocavernosus* muscle, and Cowper's gland were not affected by treatment with BHA alone. The relative testosterone propionate-stimulated ventral prostate weight was significantly increased by the 250 mg/kg BHA dose, but the absolute and formalin-fixed weight was not significantly changed.

The anti-estrogenic activity of BHA was evaluated in immature female rats.[24] Twenty-day-old, female Sprague-Dawley rats (11/group) received subcutaneous injections of 0, 50, 100, 250, or 500 mg/kg BHA, in corn oil, once per day for 3 consecutive days. To investigate anti-estrogenic activity, the injection of 50 or 500 mg/kg BHA was followed by an injection of 2 μg/kg E_2 (in corn oil), administered within 10 min for 3 d. Six hours after the last treatment, rats were weighed, and liver and uteri were extracted and prepared for histopathological analyses. Significant decreases in body weight gains were observed in the 250 and 500 mg/kg BHA-only groups. Relative liver weight was significantly increased in the 500 mg/kg BHA-only group. Relative and absolute uterine weights containing fluids were significantly decreased by all doses of BHA

alone, which were not dose-dependent; the 500 mg/kg BHA dose also significantly decreased E_2-stimulated increase of relative and absolute uterine and vaginal weight. BHA treatment did not affect uterine epithelial height, either alone or with E_2 treatment.

The potential endocrine disruptive effects of BHA were evaluated in female Wistar rats.[25] Immature (17-21 d) female Wistar rats (10/group) received 300 mg/kg bw BHA, in sunflower oil, via gavage for 3 d. A 20 µg/kg dose of E_2, in sunflower oil was administered via subcutaneous injection to positive controls. Rats were weighed and killed using diethyl ether; the genital tract, liver, spleen, and kidneys were removed, weighed, and prepared for histopathological analyses. Except for negative and positive controls, no significant changes in body weight were observed in treated groups. Rats treated with BHA had minimal histopathological changes in the uterus, with no changes in the cervix and vagina. BHA, butylated hydroxytoluene, and propyl gallate had the ability to decrease the relative uterine weight; propyl gallate had the most severe effect. Though not statistically significant, an increase in endometrial epithelium cell height was observed with BHA treatment, suggesting a possible estrogenic effect.

GENOTOXICITY STUDIES

In Vitro

BHA at 0.0075% had no mutagenic activity against Salmonella typhimurium TA1535, TA1537, TA1538, or Saccharomyces cerevisiae (D4) when tested in a series of in vitro assays, with and without metabolic activation.[2] Similar results were obtained with an analogous assay system, in which BHA was tested at 10, 100, 1000 µg/plate using S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538, with and without metabolic activation. No significant aberrations in the anaphase chromosomes of human embryonic lung cultures were observed when treated with BHA in isopropyl alcohol at concentrations of 2, 20, or 200 µg/ml. It was confirmed by a recombination assay that "deoxyribonucleic acid (DNA) damaging activities " were formed in the reaction mixture of sodium nitrite and BHA under gastric pH conditions. The active agent in the nitrite-BHA reaction system was subsequently identified as 2-tert-butyl-quinone, which was non-mutagenic in an Ames test using S. typhimurium strains TA98 and TA1535. On the other hand, 2-tert-butyl-hydroquinone, a degradation product resulting from BHA exposure to ultraviolet (UV) radiation, was mutagenic in assays with wild and recombination-less strains of Bacillus subtilis and wild and rad mutant strains of yeast. A 0.2 mM dose of BHA was found to be a "potent" enhancer of nitrous acid mutagenesis of duplex DNA in Haemophilus influenzae. Chromosomal aberration tests conducted on Chinese hamster fibroblasts in vitro were negative for 10^{-4} M BHA in ethanol.

BHA (1 – 1000 µg/plate) was not mutagenic in S. typhimurium strains TA97, TA100, TA102, or TA104, with or without metabolic activation in an Ames test.[12] However, cytotoxic effects were seen at 500 and 1000 µg/plate. BHA (0.5 – 250 µg/plate) was not mutagenic in S. typhimurium strains TA97, TA98, TA100, or TA102, with or without metabolic activation. However, lethal cytotoxic effects were seen at 100 µg/plate without metabolic activation and at 250 µg/plate with metabolic activation. In the presence of metabolic activation, 125 µg/plate BHA induced chromosomal aberrations in Chinese hamster fibroblast cells. BHA was negative for genotoxicity in the hepatocyte primary culture/DNA repair test (0.01 – 1 µg/ml, toxic at 5 µg/ml); Salmonella/microsome mutagenesis test (1 – 100 µg/plate); adult rat liver cell/HGPRT mutagenicity test (60 – 90 µg/ml); and a Chinese hamster ovary/sister chromatid exchange test (5 – 50 µg/ml, toxic at 500 µg/ml). BHA was treated with simulated gastric conditions and the resulting metabolites were tested in a Salmonella mutagenicity assay; BHA was considered capable of producing both mutagenic and anti-mutagenic metabolites. Human lymphocytes were treated with 50 or 100 µM BHA in an oxidative DNA damage assay. At 50 µM, BHA induced a dose-dependent increase in cell proliferation of phytohemagglutinin-stimulated lymphocytes; 100 µM BHA did not induce oxidative DNA damage. t-butylquinone, at 100 µM, and 50 µM tert-butylhydroquinone (both metabolites of BHA), increased formation of 7-hydroxy-8-oxo-2'-deoxyguanosine in human lymphocytes. When tested in BALB/3T3 mouse embryo cells, BHA (5 – 20 µg/ml) dose-dependently enhanced the cell transformation activity of 3-methylcholanthrene; however, cell transformation did not increase in the absence of another initiator or promoter. BHA did not increase cell proliferation.

In an Ames test, 3-BHA in DMSO, was not genotoxic at concentrations up to 1000 µg/plate to *S. typhimurium* strains TA97, TA100, TA102, and TA104, with or without metabolic activation.[13] Similarly, in another Ames test, 3-BHA, in DMSO, was not genotoxic at concentrations up to 1250 µg/plate to *Escherichia coli* WP2 strain, with metabolic activation.[13]

In Vivo

BHA did not induce mutations in a host-mediated assay when tested against S. typhimurium (TA1530 and G-46) at doses of 15, 150, or 1500 mg/kg.[2] However, significant increases in recombinant frequencies occurred at each of these concentrations in a host-mediated assay involving S. cerevisiae (D3).

BHA, administered orally at doses of 15, 150, or 1500 mg/kg in isopropyl alcohol for 5 d was non-mutagenic in a dominant lethal study with rats. In a cytogenetic study, no significant aberrations of bone marrow metaphase chromosomes were noted in rats administered either a single oral dose or 5 oral doses of 15, 150, or 1500 mg/kg BHA (each given 24 h apart). The ingestion of 0.01 – 0.15% BHA in 5% ethanol or 1% sucrose solution by Drosophila melanogaster yielded no higher frequency of sex-linked recessive lethals in mature spermatozoa than in control flies. BHA was also found to be non-mutagenic in another sex-linked recessive lethal test when fed over a 72-h period to Drosophila melanogaster at concentrations of 5% in a carrier compound of butter and 2% glucose.

The protective effect of BHA in vivo against mutagenesis by benzo[a]pyrene and other polycyclic hydrocarbons may be due to its ability to reduce levels or inhibit the formation of mutagenic metabolites. The antimutagenic effect of dietary BHA may have important exceptions. A higher activation of beef-extract mutagens in CD-1 mice following the addition of 0.75% BHA to the diet was observed.

CARCINOGENICITY STUDIES

Dermal

In a dermal carcinogenesis study, groups of 100 C3H/Anf mice (50/sex) were given weekly skin applications of either 0.1 or 10 mg BHA in acetone.[2] No gross or microscopic evidence of skin tumor formation was observed after 323 to 519 d. In another study, 1 mg BHA in acetone solution was topically applied to the shaved backs of 30 female CD-1 Charles River mice twice weekly for 30 wk. No papillomas or carcinomas were detected during weekly histologic examination or by the end of the experimental period.

Oral

Rats were given diets containing BHA at levels of either 0, 1.35, or 67.5 mg/kg in the diet for 1 yr.[2] Two fibroadenomas were noted in 13 female rats on 1 of 4 diets at the higher level. Rats given diets containing 0, 0.003, 0.03, 0.06, or 0.12% BHA for 21 to 22 mo had no tumors or other pathologic changes. No tumors were reported in rats fed diets containing 0, 0.01, or 0.1% BHA for 2 yr. In a 2-yr study, BHA was incorporated into the diet of F344 rats at 0.5 or 2%; the actual dietary concentrations were deemed to be 0.24 and 1.07%, respectively. In both males and females of the high-dose group, an increased incidence of papillomas and squamous cell carcinomas of the forestomach were observed. Benign and malignant tumors were found in other organs of BHA-treated rats, but their incidence was not significantly different from that of controls. Survival, behavior, red and white blood cell counts, and urinalysis of BHA-exposed animals were similar to controls. BHA-treated rats also showed a dose-related chronic interstitial nephritis and a lowered incidence of bile duct proliferation. Dietary administration of BHA to dogs at doses of 0, 5, 50, or 250 mg/kg/d for 15 mo, or at concentrations of 0, 0.001, 0.01, 0.1, or 0.3% for 1 yr did not cause carcinogenic effects.

Hyperplasia of the forestomach was induced in rats that received a diet containing 1% BHA for 1 wk.[12] Co-treatment with the antioxidants α-tocopherol, ellagic acid, propyl gallate, ethoxyquin, sodium L-ascorbate, or 3,3'-thiodopropionic acid increased the induction of hyperplasia. The researchers suggested that the induction of hyperplasia in rats by BHA may not be related to a free radical reaction. F344 rats fed a diet containing 12,000 ppm BHA for 110 wk developed papillomas and mild to moderate hyperplasia of the squamous stomach and mild to moderate dysplasia of the glandular stomach.

BHA was mixed in rodent diet and fed to B6C3F1 mice, F344 rats, and Syrian golden hamsters for 104 wk (n = 150 animals/species/group). Mice were given feed containing 0, 0.5, or 1% BHA, while rats and hamsters were given feed containing 0, 1 or 2% BHA. BHA caused dose-dependent increases in the incidence of forestomach hyperplasia, papilloma, and squamous cell carcinoma. Sensitivity to these effects by species was as follows: hamsters > rats > mice. In another study, the induction of hyperplasia and neoplastic lesions in the forestomach of Syrian golden hamsters by 1% crude BHA was evaluated via histopathologic and autoradiographic analyses. Severe hyperplasia developed from wk 1 in hamsters fed crude BHA, which reached a maximum level in wk 4 of 5.10 cm/10 cm basement membrane with crude BHA, which later decreased. Papillomatous lesions were found during wk 4 of hamsters being fed crude BHA, which increased to maximum levels in wk 16 of 0.29 cm/10 cm basement membrane.

Tumor Promotion

Dermal

A group of 30 mice had 200 nmol of 7-12-dimethylbenz[a]anthracene applied topically during a 1-wk initiation period, followed by topical application of 1 mg BHA in acetone solution twice weekly for 30 wk.[2] BHA was not considered a tumor promoter under these study conditions.

Oral

The development of lung tumors was not enhanced in A/J mice that were fed a diet containing 0.75% BHA for 8 wk, either prior to, or after, being fed urethan, benzo[a]pyrene, or dimethylnitrosamine.[12] Prior exposure to BHA partially protected animals against the tumorigenic effect of urethan and benzo[a]pyrene. Partial protection was also seen in animals given benzo[a]pyrene and then exposed to dietary BHA.

Other Routes

Intraperitoneal injection of the two isomers of 3-BHA and 2-BHA (failed to enhance lung tumor development).[12] The researchers concluded that BHA is not a promoting agent for lung tumors in mice.

ANTI-CARCINOGENICITY STUDIES

The BHA-mediated protection of rodents against the neoplastic effects of carcinogens is considered to be nonspecific with respect to the chemical nature of the carcinogen, the route of carcinogen administration, or the site of tumor formation.[2] Proposed mechanisms of the anti-carcinogenic effect of BHA include (1) alteration of the carcinogen metabolism by decreased activation, increased detoxification, or both, (2) scavenging of active molecular species of carcinogens to prevent

their reaching critical target sites in the cell, (3) alteration of permeability or transport, and/or (4) competitive inhibition. It has been suggested that BHA may be metabolized by mixed function oxidases in the same manner as the carcinogens, thereby allowing BHA metabolites to compete with ultimate carcinogens for the binding with cellular macromolecules.

BHA applied topically to mice inhibited the epidermally-mediated covalent binding of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene to DNA but did not significantly induce epidermal aryl hydrocarbon hydroxylase activity. The researchers suggested that BHA has an indirect effect on the epidermal metabolizing system, which leads to a decrease in the covalent binding of carcinogen to DNA. It has been reported that BHA (100 µM) can inhibit cytochrome P-450 and other hemoprotein-catalyzed oxidation of drugs and carcinogens. Other studies have shown that BHA exhibits pronounced effects on enzymes involved directly or indirectly in the metabolism of carcinogens. BHA was not effective in inhibiting 7,12-dimethylbenz[a]anthracene-induced mammary tumors when rats were fed diets containing 0.7% BHA (along with either 20% corn oil, 18% coconut oil plus 2% linoleic acid, or 2% linoleic acid), suggesting that the effectiveness of BHA as a tumor inhibitor may be altered by dietary factors.

BHA inhibited the binding of 2-acetylaminofluorene to DNA in calf thymus and rat hepatocyte cultures.[12] BHA, at 0.8, 8, 80, and 160 µM, inhibited N-acetyltransferase activity in PC-3 human prostate tumor cells in a dose-dependent manner. At 8, 80, and 160 µM, BHA was a noncompetitive inhibitor of N-acetyltransferase activity in Colo 205 human colon tumor cells. BHA, at 8 mM and 80 µM, inhibited the formation of 2-aminofluorene-DNA adducts in human prostate tumor and human colon tumor cells, respectively.

The antioxidant activity of 0.25 or 0.5 mM BHA protected Chinese hamster V79 cells from the mutagen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The higher concentration of BHA was cytotoxic. BHA did not prevent MNNG-induced DNA strand breaks, but it did not prevent their rejoining. When mice were fed a diet containing 0.7% BHA, the activity of and mRNA for peroxiredoxin I (MSP23) in the liver and small intestine was induced. The MSP23 enzyme may be important to protect cells and tissues against toxic electrophiles and reactive oxygenated species.

Female ACI rats ($n \geq 10$/group) were treated with cholesterol (controls), BHA, 17β-estradiol (E_2), or a combination of 17β-estradiol and BHA for 7, 15, 120, or 240 d.[26] Mammary tumor development was monitored twice weekly during treatment. Rats in the E_2 and the combined E_2 + BHA groups were implanted subcutaneously with pellets of 3 mg E_2 combined with 17 mg cholesterol. Control and BHA only groups had pellets containing only cholesterol implanted. E_2 and control group animals were fed a purified phytoestrogen-free diet, while E_2 + BHA and BHA only groups were fed a diet containing 0.7% BHA (w/w). Upon completion of treatment, animals were killed and mammary tissues/tumors as well as liver, uterus, kidney, lung, brain, and ovary tissues were obtained for histopathological analyses. Total 8-isoprostane $F_{2\alpha}$ levels, a marker of oxidant stress, and the activity of other antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) were quantified in the mammary and liver tissue. Neither cholesterol control nor BHA-treated groups developed mammary tumors. Tumor latency was significantly increased in the E_2 + BHA group, which exhibited a 24% incidence after 8 mo of treatment compared to the E_2 only group which exhibited the first palpable tumor after 128 d of treatment, and had an 82% tumor incidence after 8 mo. Histopathological examination of mammary tissue from the control and BHA groups revealed normal structure. No differences in tumor morphology between E_2 or E_2 + BHA-treated animals were observed, with the exception of more invasiveness in the mammary tumors resulting from E_2-treatment. No evidence of tumor or dysplasia was observed in non-target organs. Mammary tissue from rats treated with E_2 + BHA for 120 and 240 d displayed significantly lower levels of 8-isoprostane $F_{2\alpha}$ (suggesting inhibition of E_2-mediated increases). Superoxide dismutase activity was significantly reduced in rats exposed to E_2 + BHA for 240 d, compared to the E_2-treated group. No significant differences were observed in the activities of glutathione peroxidase and catalase enzymes in mammary tissue, or in superoxide dismutase, glutathione peroxidase, and catalase in the liver tissues of E_2 + BHA and BHA only groups.

OTHER RELEVANT STUDIES

Immunomodulatory Effects

The immunomodulatory effects of BHA were evaluated using male BALB/c mice.[27] Mice ($n = 10$/group) received no treatment (controls), olive oil (vehicle controls), or 100 or 200 mg/kg BHA in olive oil, via gavage, for 3 wk. At the end of treatment, animals were weighed, blood was drawn, and spleen samples were isolated and weighed individually. No effect on body, liver, or spleen weight of the mice after oral treatment with BHA was observed. BHA promoted T-cell levels and decreased B-cell levels, but did not significantly affect monocyte and macrophage levels, compared to controls. Macrophage phagocytosis and natural killer (NK) cell cytotoxicity are involved in the immune response of animals exposed to antigens. BHA was found to promote phagocytosis of macrophages from peripheral blood mononuclear cells; this effect was not observed in macrophages from the peritoneal cavity. BHA-treatment did not alter the cytotoxicity of NK cells.

Hepatic Effects

Induction of drug-metabolizing enzymes by BHA is often accompanied by liver enlargement.[2] The Select Committee on GRAS Substances reported that the enlargement of the liver and stimulation of microsomal drug-metabolizing enzymes observed with BHA are produced by at least 200 compounds of extremely diverse pharmacologic activities. Referring to a number of studies, the Committee suggested that liver enlargement (also referred to as "work hypertrophy," "physiological

overworking," or "hyperfunctional enlargement") is an adaptive response. It was noted that at levels at which BHA induces liver hypertrophy, there is no evidence of persistent hepatotoxicity.

BHA (600 – 800 mg/kg/d) administered in the diet to female Swiss-Webster mice prevented hepatotoxicity induced by 600 mg/kg acetaminophen given intraperitoneally.[12] The rate of acetaminophen elimination from the blood was increased by its glucuronidation; hepatic UDP-glucuronosyltransferase activity and hepatic UDP-glucuronide concentrations were increased by BHA. Hepatic enzymes CYP1B, glutathione S-transferase, γ-glutamylcysteine synthetase, and quinone oxidoreductase were induced in male Sprague-Dawley rats which received 0.75% BHA in the diet for 3 d.

Hormonal Effects

Microsomal fractions from bovine seminal vesicles were used to investigate the effects of various antioxidants on prostaglandin biosynthesis.[2] Concentrations of 3.08 and 6.70 μM BHA produced a 50% inhibition of prostaglandin E_2 and prostaglandin E_1, respectively. In vitro, 1.06 μM BHA inhibited prostaglandin E_2 biosynthesis by 28% and stimulated prostaglandin E_1 biosynthesis by 34%. A concentration-dependent inhibition of prostaglandin production was observed in slices of rat renal medulla treated with 1 mM BHA, but not with 0.01 or 0.1 mM BHA. Arginine vasopressin-mediated increases in cAMP were also blocked by exposure to 1 mM BHA; direct inhibition of medullary adenylate cyclase or a toxic effect were possible explanations for these phenomena. BHA was reported to stimulate prostaglandin biosynthesis (as indicated by cyclooxygenase activity) at concentrations of 30 μM but inhibited biosynthesis at 160 μM. No alteration of pituitary gonadotrophic hormone was observed in rats or guinea pigs fed BHA at a dose of 0.4 mg/kg/d for 6 mo. Inhibition of bradykinin activity was noted in isolated rat uterine horn muscle (treated with 10^{-6} M BHA) and isolated guinea pig ileum smooth muscle (treated with concentrations as low as 8×10^{-9} mole/l BHA).

The calculated whole-body exposure to BHA (1.06 μM), based on a 64 kg human adult consuming 0.1 mg/kg/d of food antioxidant, was considered to have the potential to profoundly affect prostaglandin synthesis in vivo. However, it was noted that this concentration of BHA might never be reached in the glands responsible for prostaglandin synthesis.

Effect on Melanocytes

BHA concentrations of 5×10^{-3} M were toxic to cultured guinea pig melanocytes, whereas BHA at 5×10^{-6} M caused no melanocyte damage.[2] BHA (1×10^{-2} M) reduced cytotoxicity to pig melanocytes exposed simultaneously to p-hydroxyanisole. However, BHA acted synergistically with p-hydroxyanisole to increase cellular damage as the BHA concentration decreased from 10^{-2} M to 10^{-6} M. No depigmentation was observed when BHA at concentrations of 0.1 – 1 M in various solvents was applied to the skin of 2 -5 guinea pigs each weekday for 1 – 6 mo, or, when similar concentrations of BHA were applied each weekday to the skin of 10 black mice for 2 – 4 mo.

Effects on Human Astrocytes

The effects of BHA on normal human astrocyte cell growth and the underlying mechanisms were evaluated in NHA-SV40LT cells.[28] Cells were treated with 0, 25, 50, or 100 μM BHA, and evaluated in assays measuring cell proliferation, cell cycle, and cytosolic calcium influx. Cell proliferation was 40% for cells treated with 100 μM BHA, compared to 100% growth in vehicle control-treated cells. Additionally, the percentage of cells in the sub-G1 phase increased after 100 μM BHA-treatment. BHA treatment gradually increased the cytosolic levels of calcium in human astrocytes to 160%, compared to an increase of 245% in response to the positive control, ionomycin. Moreover, treatment with BHA increased the expression of endoplasmic reticulum stress proteins, including IRE1α (inositol-requiring enzyme-1), GADD153 (growth arrest and DNA damage-inducible gene 153), and GRP78 (glucose-regulated protein 78), and the phosphorylation of eIf2α (eukaryotic initiation factor 2) in a dose-dependent manner. Based on these results, the researchers posited that BHA inhibits cell proliferation and growth of human astrocytes, induces apoptosis through cytosolic calcium-mediated endoplasmic reticulum stress, and increases the expression of pro-apoptotic proteins.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

A suntan preparation containing 0.1% BHA and an eye shadow and a face powder each containing 0.2% BHA were evaluated for their skin irritation potential in 3 separate tests.[2] In each study, the test formulation (0.1 ml) was applied for 24 h under occlusion to the shaved skin of 9 albino rabbits. Test sites were graded for irritation on a scale of 0 – 4 at 48 and 72 h after the initial reading. The primary irritation indices were 0.46 (suntan preparation), 0.4 (eye shadow), and 0.11 (face powder) indicating that the formulations were "minimal" or "slight" skin irritants. An eye makeup preparation containing 0.1% BHA was evaluated in another skin irritation study, according to procedures specified in 16 CFR 1500.41. A 24-h, occlusive application of the test material (0.5 g) was made to the intact and abraded skin of 6 albino rabbits. Test sites were graded for irritation upon patch removal and 48 h after this first reading; the primary irritation index was 2.75, indicating that the product was a moderate skin irritant.

Two guinea pig immersion tests were conducted to evaluate percutaneous toxicity and the dermal irritation potential of 2 different bubble bath formulations, each containing 0.1% BHA. Guinea pigs in each study had their abdominal hair clipped and were immersed up to their axillae in a 0.5% aqueous solution of the product. Twelve animals (6 animals/

product) were exposed to an actual BHA concentration of 0.005% (0.005 x 0.1%) 4 h/d for 3 consecutive days. Skin reactions on the abdomen were graded on a scale of 10 (normal skin) to 1 (moribund due to skin injuries) 48 h following the last exposure. The average irritation indices for the 2 formulations were 7.9 and 5, indicating mild (moderate scaling, no loss of skin elasticity), and moderate (cracking and fissuring, considerable loss in skin elasticity) skin irritation, respectively. No evidence of systemic toxicity was observed in either test.

Human

A face powder, blusher, and eye shadow formulation , each containing 0.2% BHA, were considered to cause minimal skin irritation in 3 separate 24-h patch tests conducted in 20 subjects each.[2] No skin irritation was observed in a 24-h patch test using a suntan preparation containing 0.1% BHA, performed in 20 subjects. A bubble bath formulation containing 0.1% BHA produced a primary irritation index score of 0.95 and was considered a minimal/mild irritant to skin in a 24-h patch test done in 19 subjects. Four separate 21-d, cumulative skin irritation studies were conducted to evaluate various cosmetic formulations containing BHA. The composite total score for a liquid makeup formulation containing 0.01% BHA was 148/630, suggesting slight skin irritation, when tested in 10 subjects. A polish remover containing 0.1% BHA was "essentially nonirritating"(composite score = 26/756) when tested in 12 subjects. In a third 21-d cumulative skin irritation test, 3 different skin creams, each containing 0.02% BHA were tested on 10 subjects under both occlusive and semi-occlusive conditions. Baby oil was used as a low-irritation reference; positive controls were not used. The total composite scores for cream A, cream B, cream C, and baby oil were the following, under occlusive conditions, respectively: 547/630; 411/630, 227/630, and 18/630. The total composite scores for cream A, cream B, cream C, and baby oil were the following under semi-occlusive conditions, respectively: 338/630, 208/630, 200/630, and 28/630. All scores fell within the skin irritation category "possibly mild in normal use," with the exception of scores for cream A applied under occlusion (547/630: "an experimental cumulative irritant") and baby oil ("a mild material"). Three different cosmetic pastes, tested in 10 subjects, were considered "essentially non-irritating" to very slightly irritating in another cumulative skin irritation test. The skin-irritating effects of an eye makeup preparation containing 0.1% BHA was evaluated in 51 subjects; no irritation was noted when the test material was applied in the eye area under normal use conditions for 4 wk.

Sensitization

Human

Three different shave cream formulations each containing 0.1% BHA were studied for their ability to induce primary skin irritation and sensitization in 50, 54, and 57 adult subjects, respectively.[2] In each study, panelists received a total of 8 occlusive 12-h patches containing the test material over 2 wk, followed by a 2-wk rest period, and a 24-h challenge patch. Skin reactions throughout the induction phase to 2 of the products ranged from no irritation to slight or well-defined/moderate erythema; challenge readings for both products were negative, with the exception of 1 slight erythema reaction in 1 product which occurred at the 24-h reading. Skin reactions to the third product during the induction phase ranged from no skin erythema to severe erythema and edema. Reactions ranging from no erythema to moderate erythema were noted during challenge; these reactions were not considered evidence of sensitization potential. The skin irritation and sensitization potential of a skin lightener containing 0.02% BHA was evaluated in a human repeated insult patch test (HRIPT) using 90 subjects. The first 2 induction patches contained the undiluted test material; however, subsequent induction and challenge patches contained a 50% aqueous dilution of the product. During the induction phase, 68/90 subjects showed minimal to mild irritation, whereas 22/90 exhibited no skin reactions. Twenty-four and 48 h after removal of the challenge patch, 22/90 and 8/90 subjects showed minimal to mild skin erythema, respectively. A cream containing 0.2% BHA was "essentially non-irritating" and caused "no evidence of sensitization" in a modified Draize HRIPT conducted in 108 subjects. An HRIPT was conducted with a skin freshener containing 0.05% BHA on 104 subjects; no reactions to induction or challenge applications were observed. A repeat insult maximization test was conducted on 26 subjects with a liquid makeup containing 0.01% BHA. Subject forearms were pre-treated for 24 h with an aqueous solution containing 5% sodium lauryl sulfate (SLS). No reactions were observed at challenge test sites which were not pre-treated with SLS; the researchers concluded "no evidence of contact sensitization."

Photosensitization

Human

A Draize-Shelanski HRIPT and an ultraviolet (UV) exposure method was used to evaluate a cosmetic paste containing 0.1% BHA for primary skin irritation, skin sensitization, and photosensitization in 45 subjects.[2] A pair of 1 open and 1 closed 48-h induction patches were applied every other day for 3.5 wk for a total of 10 induction applications. After a 14-d rest, 1 open and 1 closed patch were applied for a 48 h-challenge application. Closed patch sites were irradiated with UV light following removal of the first, fourth, seventh, tenth, and eleventh (challenge) insults. One "doubtful" reaction was observed in 1 individual following the second closed induction patch, and a similar reaction was observed in another subject following the eighth closed induction patch. No other reactions were observed following any of the 48-h open or closed challenge patches, nor after UV exposure. In a similar study testing a polish remover containing 0.01% BHA, a "weak" non-vesicular skin reaction was observed in 1 subject following the second of 10 closed induction patches and in 2 other subjects after the sixth closed induction patch. No other reactions to the polish remover were noted during induction or challenge insults or following UV exposure.

A Schwartz-Peck prophetic patch procedure with UV exposure was used to determine the skin-irritating, skin-sensitizing, and photosensitizing effects of a cosmetic paste containing 0.01% BHA in 110 subjects. One individual showed a "weak" non-vesicular skin reaction to the first of 2 closed patches; all other results following open and closed insults and UV exposure were negative. No evidence of skin irritation, skin sensitization, or photosensitization was observed in a similar study in which 101 subjects were exposed to a polish remover containing 0.01% BHA. In a third Schwartz-Peck patch test, 728 subjects were tested with an eye makeup formulation containing 0.1% BHA. Two "weak" non-vesicular reactions were observed after the first of 2 closed patches, whereas 4 similar reactions were observed following the second closed patch. The product was not considered an irritant, sensitizer, or a photosensitizer. The same eye makeup preparation (0.1% BHA) was evaluated in a Shelanski and Shelanski HRIPT with UV exposure. "Weak" non-vesicular reactions were observed in some subjects after the first 8 of 10 closed induction patches. Several subjects exhibited "strong" edematous and/or vesicular skin reactions observed following the closed challenge patch. Exposure to UV radiation resulted in single "weak" non-vesicular reactions following the first closed induction patch and following the challenge patch. No reactions were observed to open patches; the product was not considered an irritant, sensitizer, or a photosensitizer. BHA in 50% anhydrous alcohol at a level of 100 mg/ml conferred moderate skin protection against UV radiation in 25 subjects exposed to the equivalent of 3 minimal erythema doses.

OCULAR IRRITATION STUDIES

Animal

In 2 separate studies, the ocular irritation potential of a face powder containing 0.2% BHA and an eye shadow containing 0.2% BHA was evaluated in 6 albino New Zealand rabbits.[2] A single 0.1 ml dose of the test material was instilled into 1 eye of each animal; details on rinsing were not provided. Untreated eyes served as controls. In the second study, the test material was instilled 3 times a day (details on rinsing not provided) for an unspecified number of days into the conjunctival sac of 1 eye of each animal by means of a 4-sec spray held 6 in from the face. The researchers considered the face powder to be a "minimal" eye irritant (average eye irritation scores at 24 and 48 h following exposure were 2 and 0, respectively) and the eye shadow to be a "mild" eye irritant (average eye irritation scores on days 1, 2, 3, 4, and 7 were 2, 1, 2, 1, and 0, respectively). In a third eye irritation test, a single, 0.1 ml application of an eye makeup preparation containing 0.1% BHA was placed into 1 eye each of 6 albino rabbits; untreated eyes served as controls. Treated eyes remained unrinsed. Eyes were graded for ocular reactions 24, 48, and 72 h following instillation of the test preparation. All treated eyes were negative for conjunctival redness, conjunctival chemosis, keratitis, and iritis.

CLINICAL STUDIES

Case Reports

The North American Contact Dermatitis Group (NACDG) reported the incidence of sensitization among 548 subjects exposed to 2% BHA to be 11 subjects.[2] A 52-yr-old woman developed contact dermatitis of the face after using a cosmetic formulation containing 0.005% BHA. BHA (0.1% in soft paraffin) was identified as the offending allergen in a subsequent patch battery test; after avoiding the product, the patient ceased to have further issues. A 32-yr-old woman acquired dermatitis following use of a hand cream formulation; BHA proved to be the causal agent of her allergic dermatitis. A 48-yr-old male cook developed contact dermatitis of the hands after contact with mayonnaise containing BHA. Patch tests with the mayonnaise were positive for 2% BHA in the patient and negative in 3 controls; symptoms ceased with the avoidance of mayonnaise. One case of contact sensitivity resulted from the use of an antimycotic cream containing 0.052 mg BHA. Subsequent patch test results were positive for both 5% BHA in petrolatum and the cream's active ingredient (miconazole nitrate). A lymphocyte-mediated allergy was demonstrated in study in which patients had been sensitized after repeated local application of the chemical. Patients (n = 112) were referred to a clinic for eczematous dermatitis, of various types, and caused by various creams. When patch tested with 2% BHA in petrolatum, 3 of these patients were positive for contact dermatitis; biopsy results and control patch tests confirmed that the reactions were allergic and not a result of irritation. Eighty-three "consecutive" patients with eczematous dermatitis were patch tested with 5% BHA in alcohol; all were negative for contact dermatitis. Seven patients with suspected sensitivity to BHA showed exacerbated signs of allergy when given oral doses of 125 – 250 mg BHA following 12 h of fasting. Symptoms included chronic nasal blockage, frequent nasal polyps, chronic vasomotor rhinitis, headaches, asthma, flushing, suffusion of the conjunctivae, occasional retrosternal pain radiating to the back, somnolence, and marked diaphoresis. Increased bleeding times of 100% or more also occurred in BHA-sensitive patients after oral challenge, but not in controls. BHA-intolerance has been reported in other studies as well (n = 37 subjects). Two patients had dyshidrotic eczema which cleared when placed on a BHA-free diet. Subsequently, these patients developed vesicles on their hands and lips within 12 h of being orally challenged with BHA. Daily oral administration of 5 or 10 mg BHA for 4 d caused a flare-up of skin dermatitis in BHA-sensitive individuals. A 32-yr-old patient reacted with generalized urticaria in a double-blind study following ingestion of BHA. The patient had persistent cryoglobulinemia that did not change with "challenge" and normal baseline histamine concentrations that elevated with challenge to BHA.

Two patients had contact dermatitis reactions to pharmaceutical-grade BHA (2%) in a cream.[12] However, the 2 patients had negative reactions to analytical-grade BHA (2%) in patch tests. The researchers could not explain the discrepancy. Seven patients who had allergic contact dermatitis to some cosmetics and toiletries were found to be sensitive

to 1% BHA. Two elderly patients developed eczema after applying a topical cream treatment for psoriasis. Patch tests showed positive reactions with 2% BHA, an ingredient in the topical cream.

Cohort Study on Dietary Exposure to BHA

The association between dietary intake of BHA and BHT and stomach cancer risk was investigated in 120,852 men and women, aged 55 – 69 yr, over 6.3 yr, in the Netherlands Cohort Study.[29] Incident stomach cancer cases totaled 192 during the course of the study. A semi-quantitative food questionnaire was used to assess food consumption, and information on BHA and BHT content of cooking fats, oils, mayonnaise, other creamy salad dressings, and dried soups was obtained via chemical analyses and Dutch food additive databases. The mean intake of BHA was 105 µg/d and a statistically non-significant decrease in stomach cancer risk was observed with increasing BHA intake (rate ratio highest/lowest intake of BHA = 0.57; 95 % CI: 0.25 – 1.30). No significant association with stomach cancer risk and consumption of BHA was observed.

EXPOSURE ASSESSMENT

BHA is an authorized food additive in the EU with an ADI of 1.0 mg/kg bw/d, established by the EFSA Panel on Food Additives and Nutrient Sources added to Food.[11] Hence, for an adult weighing 60 kg, the permissible daily intake is 60 mg (= 60,000 µg). Daily exposure from BHA usage across various categories/types of cosmetic products is presented in Table 4. These conservative exposure estimates are significantly below the ADI limit for BHA (highest estimate, based on the highest reported concentration of use, is 0.45 mg/d BHA, in other manicuring preparations).

SUMMARY

BHA is reported to function in cosmetics as an antioxidant and a fragrance ingredient. The Panel first reviewed the safety of this ingredient in a safety assessment that was published in 1984; during this initial review the Panel issued a final report with the conclusion that BHA is safe as a cosmetic ingredient in the present practices of use. The Panel also previously considered a re-review of this report and reaffirmed the 1984 conclusion, as published in 2006. In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. At its June 2023 meeting, the Panel determined that this safety assessment should be reopened to explore the possible endocrine and reproductive effects of BHA at high doses and to provide an updated assessment of this ingredient.

According to 2023 VCRP survey data, BHA is reported to be used in 70 formulations; at the time of the previous rereview of this ingredient, BHA had 1224 uses reported in 2002. Results from a 2023 concentration of use survey conducted by the Council indicate that the highest reported maximum concentration of use for BHA is at 0.15% in other manicuring preparations; BHA was reported to be used at 0.2% in several product formulations (cologne and toilet waters, perfumes, blushers, and lipsticks) in 2003.

In a toxicokinetics study, rats were fed 0.12% 3-BHA in the diet for 21 mo. The test substance was absorbed from the gut via passive diffusion; no evidence of tissue storage was observed in the animals. In a human study, most of a single, oral dose of 0.5 mg/kg 3-BHA administered to human subjects was recovered in the urine and feces (95%). 3-BHA was excreted in its conjugated form in the urine and as conjugated *tert*-butyl hydroquinone in the feces. No free BHA was found in the urine or feces.

The LOEL for rats dosed "continuously" with 3-BHA, via gavage, for 6 wk was determined to be 63,000 mg/kg bw; in another oral toxicity study, the LOEL for rats dosed "continuously" with 3-BHA, via gavage for 16 wk was determined to be 9900 mg/kg bw. In another subchronic oral toxicity study, male C57BL/6J mice (24/group) were either fed a normal diet or a high-fat diet, prior to receiving either 0.2% DMSO, 1 or 10 mg/kg bw BHA, via gavage, for 18 wk. Patterns of inguinal subcutaneous white adipose tissue and perigonadal visceral white adipose tissue accumulation were consistent with body weight gain in BHA-treated, high-fat diet mice. Upon treatment with BHA, the mRNA levels of adipogenic transcriptional factors in perigonadal visceral white adipose tissue were significantly increased in a dose-dependent manner for both natural and high-fat diet groups ($p < 0.05$ or 0.01). Rats received 0, 0.125, 0.25, 0.5, 1, or 2% BHA in the diet for 104 wk; body weights were reduced in rats receiving at least 0.5% BHA. Significant pathology was only seen in the forestomach epithelium in animals exposed to > 0.5% BHA.

The LOAEL for BHA affecting embryoid body morphogenesis in P19C5 stem cells was 400 µM; BHA influenced the expression of developmental genes in a temporal and gene-specific manner. Groups of male and female Sprague-Dawley rats (12/sex/group) were given up to 500 mg/kg BHA, in corn oil, via gavage, in a generational developmental and reproductive toxicity study. The weights of liver, adrenal and thyroid glands were increased, mating rate was decreased, and cohabitation during conception was longer in the F_0 generation exposed to 500 mg/kg BHA. F_1 rats exposed to 500 mg/kg BHA exhibited significant reductions in the body weights at postnatal day 21, increased liver and adrenal gland weights, decreased spleen, vagina, testes, and ventral prostate weights, reduced velocity of sperm motion and number, lowered serum levels of thyroxine and testosterone, slightly shortened estrous cycle length, and effects in the follicular epithelial cells of the thyroid. In another developmental toxicity study, in which rats were dosed with up to 420 mg/kg/d BHA in the diet, the NOAEL for maternal toxicity was determined to be 420 mg/kg/d. Based on reduced weight of progeny during lactation and increasing peri-

weaning mortality, a NOAEL of 220 mg/kg/d BHA and a LOAEL of 420 mg/kg/d BHA were established for offspring toxicity.

BHA significantly downregulated the mRNA expression of genes involved in the biosynthesis of steroids in 2 separate in vitro studies using immature mouse Leydig cells and Sertoli cells and immature rat Leydig cells. BHA did not exhibit anti-androgenic activity, when tested alone in a luciferase reporter gene assay using MDA-kb2 breast cancer cells; when tested at 300 µM, in the presence of 1000 pm DHT, BHA completely inhibited DHT-induced luminescence and exhibited significantly anti-androgenic activity in a concentration-dependent manner. In another luciferase assay using PC-3 prostate carcinoma cells, the EC_{50} for BHA to inhibit DHT-mediated activation was 7.6 µM; BHA was considered a partial androgen antagonist. BHA exhibited a higher potency of estrogenic effects when tested at up to 300 µM in a luciferase assay using T47D-Kbluc breast cancer cells, in comparison to being evaluated in a proliferation assay using MCF-7 breast cancer cells The anti-androgen-, glucocorticoid-, and thyroid hormone-like activities of BHA were evaluated in multiple luciferase assays using MDA-kb2 and GH3.TRE-Luc reporter cell lines; BHA exhibited glucocorticoid and anti-androgen activities.

Groups of male Sprague-Dawley rats received up to 500 mg/kg BHA, in corn oil, via gavage for 10 d in a Hershberger assay; groups of the tested animals received a single, subcutaneous injection of testosterone propionate, dissolved in corn oil, at a volume of 0.4 ml/kg. Body weight gain was significantly affected by testosterone propionate alone and combined with 250 mg/kg BHA. Relative liver and adrenal weights were significantly increased by treatment with 500 mg/kg BHA alone; relative adrenal gland weight was significantly decreased by testosterone propionate alone and in combination with 250 mg/kg BHA. The relative and absolute weights of the ventral prostate, seminal vesicle with coagulating glands, glans penis, *levator ani plus bulbocavernosus* muscle, and Cowper's gland were not affected by treatment with BHA alone. The relative testosterone propionate-stimulated ventral prostate weight was significantly increased by the 250 mg/kg BHA dose, but the absolute and formalin-fixed weight was not significantly changed. The anti-estrogenic activity of BHA was evaluated in immature female rats that received subcutaneous injections of 0, 50, 100, 250, or 500 mg/kg BHA, in corn oil, followed by injection with 2 µg/kg E_2, in corn oil, once per day for 3 d. Significant decreases in body weight gains were observed in the 250 and 500 mg/kg BHA-only groups. Relative liver weight was significantly increased in the 500 mg/kg BHA-only group. Relative and absolute uterine weights containing fluids were significantly decreased by all doses of BHA alone, which were not dose-dependent; the 500 mg/kg BHA dose also significantly decreased E_2-stimulated increase of relative and absolute uterine and vaginal weight. BHA treatment did not affect uterine epithelial height, either alone or with E_2 treatment. The potential endocrine disruptive effect of ingesting BHA (300 mg/kw for 3 d), in sunflower oil, was evaluated in immature female Wistar rats. Positive controls received a 20 µg/kg dose of E_2, in sunflower oil, via subcutaneous injection. Except for negative and positive controls, no significant changes in body weight were observed in treated groups. Rats treated with BHA had minimal histopathological changes in the uterus, with no changes in the cervix and vagina. A non-significant increase in endometrial epithelium cell height was observed with BHA-treatment.

3-BHA, in DMSO, was not genotoxic when tested at concentrations up to 1000 µg/plate to *S. typhimurium* strains TA97, TA100, TA102, and TA104, in an Ames test, with or without metabolic activation. In another Ames test, 3-BHA tested at up to 1250 µg/plate, in DMSO, was not genotoxic to *E. coli* WP2 strain, with metabolic activation.

Female ACI rats were treated with cholesterol (controls), BHA, E_2, or a combination of E_2 and BHA for 7, 15, 120, or 240 d, via subcutaneous pellets, and a diet containing 0.7% BHA (for E_2 + BHA and BHA only groups). Mammary tumor development was monitored during treatment. Neither cholesterol control nor BHA-treated groups developed mammary tumors. Tumor latency was significantly increased in the E_2 + BHA group, which exhibited a 24% incidence after 8 mo of treatment compared to the E_2 only group which exhibited the first palpable tumor after 128 d of treatment, and had an 82% tumor incidence after 8 mo. Histopathological examination of mammary tissue from the control and BHA groups revealed normal structure. No differences in tumor morphology or enzyme activity were observed between E_2 or E_2 + BHA-treated animals, with the exception of more invasiveness in the mammary tumors resulting from E_2-treatment.

In a study examining the immunomodulatory effects of BHA in male BALB/c mice, animals received 100 or 200 mg/kg BHA, in olive oil, via gavage, for 3 wk. No effect on body, liver, or spleen weight was observed after oral treatment with BHA. BHA did exhibit an increase in endometrial epithelium cell height, which was not statistically significant. BHA was found to promote phagocytosis of macrophages from peripheral blood mononuclear cells; this effect was not observed in macrophages from the peritoneal cavity. BHA-treatment did not alter the cytotoxicity of NK cells.

Human astrocyte cells treated with 0, 25, 50, or 100 µM BHA were evaluated for effects on cell proliferation, cell cycle, and cytosolic calcium influx. For cells treated with 100 µM BHA, cell proliferation reduced to 40% and the percentage of cells in the sub-G1 phase increased. BHA treatment gradually increased cytosolic levels of calcium and the expression of endoplasmic reticulum stress and pro-apoptotic proteins.

The association between dietary intake of BHA and BHT and stomach cancer risk was investigated in the Netherlands Cohort Study. The mean intake of BHA was 105 µg/d and a statistically non-significant decrease in stomach cancer risk was observed with increasing BHA intake (rate ratio highest/lowest intake of BHA = 0.57; 95 % CI: 0.25 – 1.30). No significant association with stomach cancer risk and consumption of BHA was observed.

BHA is an authorized food additive in the EU with an ADI of 1.0 mg/kg bw/d, established by the EFSA Panel on Food Additives and Nutrient Sources added to Food. Thus, conservative exposure estimates for BHA in cosmetic products, based on the highest reported concentrations of use, are significantly below the ADI limit (0.45 mg/d BHA in other manicuring preparations).

No new acute toxicity, carcinogenicity, dermal irritation and sensitization, or ocular irritation data were found in the published literature, and unpublished data were not provided.

DISCUSSION

In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 yr. In 1984, the Panel published a final report with the conclusion BHA is safe as a cosmetic ingredient in the present practices of use (as described in the safety assessment); this conclusion was reaffirmed in September 2023, as published in 2006. A re-review was initiated at the June 2023 Panel meeting to evaluate potential endocrine and reproductive effects of BHA at high doses and to provide an updated assessment of the safety of this ingredient. Upon additional re-review, the Panel again concluded that BHA is safe in cosmetics in the present practices of use and concentration.

The Panel reiterated that both animal and human studies have shown that BHA is absorbed from the gastrointestinal tract and metabolized. Tissue storage may occur with BHA because of its lipid solubility; however, the amount stored is limited by rapid metabolism and excretion. BHA is not mutagenic, and has been shown to inhibit mutagenesis. No evidence of carcinogenicity was observed when BHA was administered by topical application to mice and orally to rats and dogs. Also, formulations containing BHA elicited, at most, minimal or moderate skin and eye irritation in rabbits. Furthermore, clinical data for BHA in cosmetic formulations indicated that the formulations were generally non-sensitizing, non-photosensitizing, and only minimally or mildly irritating.

The Panel considered the developmental and reproductive toxicity and endocrine studies presented in the updated report, and stated that any developmental and reproductive, endocrine, androgenic, and estrogenic effects that were observed were seen primarily in cell systems and at non-physiological concentrations, thus mitigating any concerns. The Panel also noted the GRAS status for use in foods in the US, and stated that the exposure assessment included in the document was useful when evaluating safety.

The Panel discussed the issue of possible incidental inhalation exposure resulting from formulations containing BHA (e.g., it is used in fragrance preparations at up to 0.001%, in spray deodorants at up to 0.000051%, and in face powders at up to 0.05%). Inhalation toxicity data were not available. However, the Panel noted that the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Therefore, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that BHA is safe in cosmetics in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Chemical properties of BHA

Property	Value	Reference
Physical Form	solid	[2]
Color	white or slightly yellow	[2]
Odor	aromatic	[2]
Molecular Weight (g/mol)	180.2 g/mol	[2]
Vapor pressure (mmHg @ 25ºC)	0.00248	[4]
Melting Point (ºC; ≥ 90% 3-BHA; ~8% 2-BHA)	57	[2]
Boiling Point (ºC @ 745 mm Hg) (°C; 85% 3-BHA; 15% 2-BHA)	269 54-58	[2]
Water Solubility (g/l @ 25ºC)	0.213	[4]
Other Solubility Soluble Insoluble	 alcohol, propylene glycol, chloroform, ether, fats and oil water	[2]
log K_{ow}	3.5	[4]

Table 2. Frequency (2023/2002) and concentration (2023/2003) of use of BHA according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[6]	**2002[3]**	**2023[7]**	**2003[3]**
Totals*	**70**	**1224**	**0.00000004 – 0.15**	**0.000004 – 0.2**
summarized by likely duration and exposure**				
Duration of Use				
Leave-On	*67*	*1160*	*0.00000004 – 0.15*	*0.0001 – 0.2*
Rinse-Off	*2*	*60*	*0.00025 – 0.0084*	*0.000004 – 0.05*
Diluted for (Bath) Use	*1*	*4*	*NR*	*0.00001 – 0.0004*
Exposure Type*				
Eye Area	21	524	0.000086 – 0.05	0.0001 – 0.1
Incidental Ingestion	2	279	0.00045 – 0.05	0.01 – 0.2
Incidental Inhalation-Spray	23[a]; 7[b]	49; 85[a]; 88[b]	0.00000004 – 0.001	0.0001 – 0.2; 0.02 – 0.06[a]; 0.004 – 0.1[b]
Incidental Inhalation-Powder	1; 7[b]	13; 88[b]; 1[c]	0.05; 0.00013 – 0.013[c]	0.0002 – 0.005; 0.004 – 0.1[b]; 0.0001[c]
Dermal Contact	57	903	0.00001 – 0.05	0.000004 – 0.2
Deodorant (underarm)	NR	1[a]	spray: 0.000051 not spray: 0.00076	0.002[a]
Hair - Non-Coloring	7	13	0.00000004 – 0.0084	0.0001 – 0.05
Hair-Coloring	1	1	NR	NR
Nail	1	10	0.15	0.001 – 0.06
Mucous Membrane	3	295	0.00045 – 0.05	0.000004 – 0.2
Baby Products	NR	1	NR	0.0001
as reported by product category				
Baby Products				
Baby Lotions/Oils/Powders/Creams	NR	1	NR	0.0001
Bath Preparations (diluted for use)				
Bath Oils, Tablets, and Salts	NR	4	NR	0.0004
Bubble Baths	NR	NR	NR	0.00001
Other Bath Preparations	1	3	NR	0.0001
Eye Makeup Preparations				
Eyebrow Pencil	NR	51	0.05	0.0001
Eyeliner	NR	399	0.05	0.1
Eye Shadow	12	38	0.000086 – 0.05	0.002
Eye Lotion	5	2	NR	NR
Eye Makeup Remover	NR	6	NR	0.02
Mascara	2	18	0.03	0.1
Other Eye Makeup Preparations	2	10	NR	0.001
Fragrance Preparations				
Cologne and Toilet Water	NR	18	NR	0.2
Perfumes	NR	6	NR	0.2
Powders (dusting/talcum, excl aftershave talc)	NR	2	NR	0.0002
Other Fragrance Preparation	NR	10	0.001	0.004
Hair Preparations (non-coloring)				
Hair Conditioner	NR	5	0.0084	0.0002
Hair Spray (aerosol fixatives)	NR	NR	0.00000004	0.0001
Shampoos (non-coloring)	NR	NR	0.0024	0.0005
Tonics, Dressings, and Other Hair Grooming Aids	6	8	NR	0.02
Other Hair Preparations	1	NR	NR	0.05

Table 2. Frequency (2023/2002) and concentration (2023/2003) of use of BHA according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[6]	**2002[3]**	**2023[7]**	**2003[3]**
Hair Coloring Preparations				
Other Hair Coloring Preparation	1	1	NR	NR
Makeup Preparations				
Blushers (all types)	NR	26	NR	0.2
Face Powders	1	11	0.05	0.005
Foundations	NR	30	0.02	0.05
Lipstick	2	279	0.05	0.2
Makeup Bases	1	4	NR	0.005
Rouges	NR	1	NR	0.04
Other Makeup Preparations	2	23	NR	0.05
Manicuring Preparations (Nail)				
Basecoats and Undercoats	NR	3	NR	NR
Cuticle Softeners	NR	2	NR	0.001
Nail Creams and Lotions	NR	1	NR	NR
Nail Polish and Enamel	NR	NR	NR	0.06
Other Manicuring Preparations	1	4	0.15	0.004
Oral Hygiene Products				
Dentifrices	NR	NR	0.00045	0.01
Personal Cleanliness Products				
Bath Soaps and Detergents	NR	5	0.0006 – 0.0022	0.000004
Deodorants (underarm)	NR	1	aerosol: 0.000051 not spray: 0.00076	0.002
Other Personal Cleanliness Products	NR	4	NR	0.002
Shaving Preparations				
Aftershave Lotion	NR	2	NR	0.006
Shaving Cream	NR	10	NR	0.0003
Other Shaving Preparations	NR	NR	NR	0.0003
Skin Care Preparations				
Cleansing	1	23	0.00025	0.05
Face and Neck (exc shave)	5	15	not spray: 0.00013 – 0.013	0.1
Body and Hand (exc shave)	2	72	not spray: 0.0021	0.1
Foot Powders and Sprays	NR	1	NR	0.004
Moisturizing	11	51	NR	0.06
Night	4	26	not spray: 0.00001	0.04
Paste Masks (mud packs)	NR	3	NR	0.004
Skin Fresheners	2	2	NR	NR
Other Skin Care Preparations	8	30	NR	0.03
Suntan Preparations				
Suntan Gels, Creams, and Liquids	NR	7	NR	0.1
Indoor Tanning Preparations	NR	1	NR	NR
Other Suntan Preparations	NR	5	NR	NR

NR – not reported

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders

Table 3. Developmental and reproductive and toxicity studies

Test Article	Vehicle	Test System/Animals	Dose/Concentration	Procedure	Results	Reference
			IN VITRO			
BHA	water	P19C5 stem cells	10, 100, or 1000 µM	Cells were treated with BHA for embryoid body analysis. Gene expression patterns for embryoid bodies treated with BHA at 600 µM were evaluated for 4 d.	Embryoid morphogenesis was observed at 400 µM for BHA. The LOAEL for BHA affecting embryoid body morphogenesis was 400 µM (reduced area by more than 20%, relative to control embryoid bodies). BHA down-regulated a pluripotency maintenance gene *Pou5f1* by day 1, which was also observed in control embryoid bodies. Transcription factor genes that regulate mesendoderm specification (*Brachyury*, *Cdx1, Mix11, Sp5,* and *Lhx1*), were strongly upregulated by day 1in control embryoid bodies; this day 1 upregulation was diminished in BHA-treated cells. Expression of genes involved in embryo elongation and pattering in the caudal end (*Wnt3a*, *Tbx6*, *Hes7*, and *Lfng*) were strongly up-regulated in controls at day 2; day 2 peak expressions of *Tbx6* and *Lfng* were significantly diminished by BHA, whereas those of *Wnt3a* and *Hes7* were not. BHA differentially affected the expression of genes that regulate axial patterning. Overall, BHA influenced the expression of developmental genes in a temporal and gene-specific manner.	[16]
			ORAL			
BHA	corn oil	Male and female Sprague-Dawley rats (12/sex/group)	0, 10, 100, or 500 mg/kg	F_0 male rats were treated for a total of 7 wk, after which they were killed for the measurement of organ weights, analysis of hormones and cholesterol in serum, examination of sperm motility, and morphology and necropsy findings. F_0 female rats were treated for a maximum of 10 wk (including 3 wk of gestation and 3 wk of lactation), and were killed for the evaluation of necropsy findings, organ weights, and hormone and cholesterol contents in serum after weaning. Twelve offspring (F_1; 1-2 pups/sex/litter) were treated with the same doses of BHA from postnatal day 21 until 13 wk old and another 12 F_1 offspring from each sex, litter, and treatment group were killed for evaluation of anogenital distance, necropsy findings, or organ weights on postnatal day 21.	For F_0 rats, weights of the liver, adrenal and thyroid glands were increased, mating rate was decreased, and cohabitation during conception was longer, in the 500 mg/kg group. For F_1 rats, body weights were significantly reduced in the 500 mg/kg group at postnatal day 21. Weights of the liver and adrenal glands were increased, while the weights of the spleen, vagina, testes, and ventral prostate were decreased in F_1 rats exposed to 100 or 500 mg/kg BHA for 13 wk. Additionally, a reduced velocity of sperm motion and number, lowered serum levels of thyroxine and testosterone, slightly shortened estrous cycle length, and effects in the follicular epithelial cells of the thyroid were observed in F_1 rats exposed to 500 mg/kg BHA.	[17]
BHA	in diet	Male and female rats	0, 110, 220, or 420 mg/kg/d	Animals were fed at least 14 d before mating and 1 – 14 d during breeding. This diet was maintained for pregnant dams during gestation and lactation, and up to 90 d for most pups.	NOAEL for maternal toxicity: 420 mg/kg/d Based on reduced weight of progeny during lactation (at 14 and 21 d of age) and increasing peri-weaning mortality (13%), a NOAEL of 220 mg/kg/d BHA and a LOAEL of 420 mg/kg/d BHA in the diets of dams and offspring was established.	[15]

F_0 – first/parental generation; F_1 – second/offspring generation; LOAEL – lowest-observed-adverse-effect level; NOAEL – no-observed-adverse-effect level

Table 4. BHA exposures from daily usage across various categories/types of cosmetic products

Product Category/ Type of cosmetics exposure	Daily Exposure by Product Category* (mg/d)	Maximum Concentration of Use (%)	Daily Exposure Based on Highest Use Concentration (mg/d)	Note
Eyebrow pencils	20	0.05	0.01	Exposure amount of eye shadow applied
Eyeliners	5	0.05	0.0025	
Eye shadows	20	0.000086-0.05	0.01	
Mascaras	25	0.03	0.0075	
Other fragrance preparations	1500[#]	0.001	0.015	Exposure amount of eau de toilette spray applied
Hair conditioners	40	0.0084	0.00336	
Hair sprays (aerosol)	5000 [γ]	0.00000004	.000002	
Shampoos (noncoloring)	110	0.0024	0.00264	
Face powders	85[†]	0.05	0.0425	
Foundations	510	0.02	0.102	
Lipstick	60	0.05	0.03	
Other manicuring preparations	300 [#]	0.15	0.45	Exposure amount of nail polish applied
Dentifrices	138	0.00045	0.000621	Exposure amount of toothpaste applied
Bath soaps and detergents	49.8	0.0006-0.0022	0.0011	Exposure amount of bath oil, salts, etc. applied
Deodorants Not spray Aerosol	 1500 6540	 0.00076 0.000051	 0.0114 0.003335	
Skin cleansing (cold creams, cleansing lotions, liquids and pads)	190	0.00025	0.000475	Exposure amount of shower gel applied
Face and neck products (not spray)	1540	0.00013-0.013	0.20	Exposure amount of face cream/lotion applied
Body and hand products (not spray)	7820	0.0021	0.0164	Exposure amount of body lotion applied
Night products (not spray)	308 [#]	0.00001	0.000031	Exposure amount of face mask applied

* Exposure parameters are retrieved from the SCCS NoG[30]

† Exposure amount is provided by Steiling et al. 2018[31]

γ Exposure amount is provided by the Consumer, Toiletry, Fragrance Association (CTFA; currently known as the Council) habits and practices data[32]

Exposure amount is provided by Vermeer Cosmolife[33]

Of note, BHA is reported to be used at concentrations up to 1% for *Other Nail Products* and 5 mg/g for *Mascara/Eyelash Products* in California Safe Cosmetics Program (CSCP) Product Database.[34]

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