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## **Safety Assessment of BHA as Used in Cosmetics**

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Status: Re-Review for Panel Consideration  
Release Date: May 19, 2023  
Panel Meeting Date: June 12 - 13, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Preethi Raj, M.S., Senior Scientific Analyst/Writer, CIR.



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### **Memorandum**

To: CIR Expert Panel Members and Liaisons  
From: Preethi S. Raj, M.Sc.,  
Senior Scientific Analyst/Writer, CIR  
Date: May 19, 2023  
Subject: Re-Review of the Safety Assessment of BHA

The Expert Panel for Cosmetic Ingredient Safety (Panel) first published a review of the safety of Butylated Hydroxyanisole (since renamed as BHA) in 1984 (identified in the pdf as *originalreport\_BHA\_062023*). On the basis of the available information 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 previously considered a re-review of this report and reaffirmed the 1984 conclusion, as published in 2006 (*rereview2006\_BHA\_062023*).

Because it has been at least 15 years since the previous re-review was published, in accordance with Cosmetic Ingredient Review (CIR) Procedures, the Panel should consider whether the safety assessment of BHA should be re-opened. An extensive search of the world's literature was performed as of April 2023 for studies dated 2001 forward. A number of studies evaluating endocrine activity as well as reproductive and developmental effects of BHA, both in vitro and in vivo, have been found. A few oral toxicokinetic studies, several repeated oral dose toxicity studies, 2 Ames tests, a study on the immunomodulatory effects of BHA in mice, another study on the anti-carcinogenicity effects of BHA in rats, and a Dutch cohort study examining the association between the dietary intake of BHA and stomach cancer risk were also found. Of note, the web-based *International Cosmetic Ingredient Dictionary* defines BHA as a mixture of tert-butylated 4-hydroxy-anisole isomers which consists chiefly of 3-tert-butyl-4-hydroxyanisole with lesser amounts of 2-tert-butyl-4-hydroxy-anisole. Thus, data found on BHA in both isomeric forms has been included (and identified). An historical overview, comparison of original and new use data, and the search strategy used are included herein (*newdata\_BHA\_062023*).

Also included for your review is a table of current and historical use data (*usetable\_BHA\_062023*). According to 2023 FDA VCRP data, BHA has 70 reported uses; at the time this ingredient was last considered for re-review, 1224 uses were reported. Reported use categories have not changed significantly and concentrations of use have remained constant over time. In 2023, the maximum reported concentration of use for BHA is at 0.15% in other manicuring preparations, while BHA was reported to be used at 0.2% in several product formulations (cologne and toilet waters, perfumes, blushers, and lipstick) in 2003.

If upon review of the updated use data the Panel determines that a re-review is warranted, a Draft Amended Report will be presented at an upcoming meeting.

**Re-Review - BHA - History and New Data**

(Preethi Raj – June 2023 meeting)

Ingredients (1)	Citation	Conclusion	Use - New Data	Results	Use - Existing Data	Results	Notes
<b>BHA</b>	JACT 3(5): 83-146, 1984	safe as used	frequency of use (2023)	70 uses	frequency of use (2002)	1224 uses	frequency of use decreased significantly
<i>Changes to Original List</i> Name updated from Butylated Hydroxyanisole to BHA	IJT 25(S2):7-10, 2006	reaffirmed	conc of use (2023)	≤ 0.15%	conc of use (2003)	≤ 0.2%	concentration of use remained constant; no new use categories

NOTABLE NEW DATA			
Publication	Study Type	Results – Brief Overview	Different from Existing Data?
<b>Non-Cosmetic</b>			
EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Scientific opinion on the re-evaluation of butylated hydroxyanisole – BHA (E 320) as a food additive. EFSA J 2011; 9(10): 2392.	Regulation, food additive	Based on a NOAEL of 100 mg/kg bw/d for growth retardation, increased mortality and behavioral effects in rat pups at higher dose levels, and using an uncertainty factor of 100, the Panel established an ADI of 1.0 mg/kg bw/d.	Yes; the ADI has increased since the last review
Bampidis et al. Safety of butylated hydroxy anisole (BHA) for all animal species. EFSA J 2019 Dec; 17(12):e05913	Regulation, animal feed additive	Maximum authorized BHA content in animal feed (all animals, except cats), either alone, or together with butylated hydroxytoluene (BHT) and/or ethoxyquin: 150 mg/kg	Yes; not reported to be used in feed additives previously
<a href="https://www.ecfr.gov/">https://www.ecfr.gov/</a> Online electronic Code of Federal Regulations (eCFR) database	Regulations, non-cosmetic use	21CFR582.3169; GRAS use in foods when the total content of antioxidants 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: <a href="https://www.ecfr.gov/current/title-21/chapter-I/subchapter-E/part-582/subpart-D/section-582.3169">https://www.ecfr.gov/current/title-21/chapter-I/subchapter-E/part-582/subpart-D/section-582.3169</a>  21CFR175.125; Approved use in pressure-sensitive adhesives, food contact materials: <a href="https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-175">https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-175</a>  21CFR175.300; Approved use as an antioxidant in resinous and polymeric coatings, food contact materials: <a href="https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-175/subpart-C/section-175.300">https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-175/subpart-C/section-175.300</a>  21CFR176.210; Approved use as a defoaming agent, food packaging materials: <a href="https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-176/subpart-B/section-176.210">https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-176/subpart-B/section-176.210</a>  21CFR179.45; Approved use as a component of polyethylene film used, in food packaging materials, during the irradiation of prepackaged foods: <a href="https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-179/subpart-C/section-179.45">https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-179/subpart-C/section-179.45</a>	Yes; although similar CFRs and approved uses are mentioned, these CFR citations are not in the original report

NOTABLE NEW DATA			
Publication	Study Type	Results – Brief Overview	Different from Existing Data?
<b>Toxicokinetics</b>			
<a href="https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988">https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988</a>	Toxicokinetics, animal, oral	Rats were fed 0.12% BHA (3- <i>tert</i> -butyl-4-hydroxyanisole), in the diet, for 21 mo. The test substance was absorbed from the gut by passive diffusion; no evidence of tissue storage was observed in the animals.	no
<a href="https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988">https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988</a>	Toxicokinetics, human, oral	Subjects (sex and number not specified) received a single, oral dose of 0.5 mg/kg BHA (3- <i>tert</i> -butyl-4-hydroxyanisole). Most of the test substance was recovered in the urine and feces (95%); BHA was excreted in its conjugated form in the urine and as conjugated <i>tert</i> -butyl hydroquinone in the feces. No free BHA was found in urine or feces.	no
<a href="https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988">https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988</a>	Toxicokinetics, human, oral	Male subjects (number not specified) received a single, oral dose of 50 mg BHA (3- <i>tert</i> -butyl-4-hydroxyanisole; vehicle was not specified). The test substance was excreted in the urine at 27 - 77%, as glucuronide and urinary metabolites.	no
<a href="https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988">https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988</a>	Toxicokinetics, human, oral	Subjects (sex and number not specified) received a single, oral dose of 0.5 mg/kg BHA (3- <i>tert</i> -butyl-4-hydroxyanisole). Most of the test substance was recovered in the urine and feces (95%); BHA was excreted in its conjugated form in the urine and as conjugated <i>tert</i> -butyl hydroquinone in the feces. No free BHA was found in urine or feces.	no
<b>Toxicity</b>			
<a href="https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988">https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988</a>	Short-term oral toxicity, animal, oral	The LOEL for rats consistently dosed with BHA (3- <i>tert</i> -butyl-4-hydroxyanisole), via gavage, for 6 wk, was determined to be 63,000 mg/kg bw. No further details were provided.	no
<a href="https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988">https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988</a>	Subchronic oral toxicity, animal, oral	The LOEL for rats consistently dosed with BHA (3- <i>tert</i> -butyl-4-hydroxyanisole), via gavage, for 16 wk, was determined to be 9900 mg/kg bw. No further details were provided.	no
<a href="https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988">https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988</a>	Short-term oral toxicity, animal, oral	The LOEL for monkeys intermittently dosed with BHA (3- <i>tert</i> -butyl-4-hydroxyanisole), via gavage, for 84 d, was determined to be 15,000 mg/kg bw. No further details were provided.	no
Sun et al. Perturbation of 3- <i>tert</i> -butyl-4-hydroxyanisole in adipogenesis of male mice with normal and high fat diets. <i>Sci Tot Environ.</i> 2020 Feb 10; 703: 135608.	Subchronic oral toxicity, animal, oral	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). Both natural diet and high-fat diet groups were randomly categorized into 3 groups (8/group) to receive either 0.2% DMSO, 1 or 10 mg/kg bw BHA (3- <i>tert</i> -butyl-4-hydroxyanisole), via gavage, for 18 wk (doses were based on the human ADI of 1 mg/kg bw/d). Rats 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 BHA group had significantly decreased body weight gain at 0.49 g/wk, while rats in the 10 mg/kg BHA 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 <i>PPAR<math>\gamma</math></i> , <i>Srebp1c</i> , <i>CD36</i> , <i>IL6</i> , and <i>TNF<math>\alpha</math></i> in perigonadal visceral white adipose tissue were significantly increased in a dose-dependent manner for both natural diet and high-fat diet groups ( $p < 0.05$ or $0.01$ ). Glucose metabolism and insulin sensitivity remained unaffected.	Yes, data on adipogenesis not in original report
US EPA 2005 Inert Ingredient Reassessment: Butylated Hydroxyanisole (25013-16-5); PDF p. 8 - 9 <a href="https://www.epa.gov/sites/default/files/2015-04/documents/bhtbha.pdf">https://www.epa.gov/sites/default/files/2015-04/documents/bhtbha.pdf</a>	Chronic oral toxicity, oral	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. (further details were not provided).	no

NOTABLE NEW DATA			
Publication	Study Type	Results – Brief Overview	Different from Existing Data?
Yuan et al. Developmental toxicity assessment of common excipients using a stem cell-based in vitro morphogenesis model. Food Chem Toxicol. 2017 Nov; 107 (Pt 1): 376 – 385.	Developmental toxicity, in vitro	The ability of 6 food excipients allura red AC, brilliant blue FCF, tartrazine, BHA (each in water), or sodium metabisulfite, and methylparaben (both in DMSO) to affect P19C5 stem cell line-derived embryoid body morphogenesis and the expression of genes involved in developmental regulation was evaluated. Cells were treated with 10, 100, or 1000 µM of each excipient for embryoid body analysis and gene expression patterns for embryoid bodies treated with BHA at 600 µM were evaluated for 4 d. The LOAEL for BHA affecting embryoid body morphogenesis was 400 µM (reduced area by more than 20%, relative to control embryoid bodies), compared to 600 µM for allura red AC and brilliant blue FCF (reduced area), and 200 µM for methylparaben (increased average elongation distortion index by more than 40%, relative to control embryoid bodies). BHA influenced the expression of developmental genes in a temporal and gene-specific manner.	Yes, data on food additives not in original report
Jeong et al. Effects of butylated hydroxyanisole on the development and functions of reproductive system in rats. Toxicol. 2005; 208: 49-62.	Developmental and reproductive toxicity, oral	Male and female Sprague-Dawley rats (12/group/sex) were administered 0, 10, 100, or 500 mg/kg BHA, in corn oil, via gavage. F <sub>0</sub> 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 <sub>0</sub> 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 <sub>1</sub> ; 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 <sub>1</sub> 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 <sub>0</sub> rats, organ 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 <sub>1</sub> rats, body weights were significantly reduced in the 500 mg/kg group at postnatal day 21. The weights of liver and adrenal glands were increased, while the weights of the spleen, vagina, testes, and ventral prostate were decreased in F <sub>1</sub> 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 <sub>1</sub> rats exposed to 500 mg/kg BHA.	no
US EPA 2005 Inert Ingredient Reassessment: Butylated Hydroxyanisole (25013-16-5); PDF p. 10 <a href="https://www.epa.gov/sites/default/files/2015-04/documents/bhtbha.pdf">https://www.epa.gov/sites/default/files/2015-04/documents/bhtbha.pdf</a>	Developmental and reproductive toxicity, oral	BHA was administered to male and female rats at 110, 220, or 420 mg/kg/d, in the diet, 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. The NOAEL for maternal toxicity was determined to be 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.	no
<a href="https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988">https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988</a>	Genotoxicity, in vitro	In an Ames test, BHA (3- <i>tert</i> -butyl-4-hydroxyanisole), in DMSO, was not genotoxic at concentrations up to 1000 µg/plate to <i>Salmonella typhimurium</i> strains TA97, TA100, TA102, and TA104, with or without metabolic activation.	no
<a href="https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988">https://echa.europa.eu/da/registration-dossier/-/registered-dossier/15988</a>	Genotoxicity, in vitro	In an Ames test, BHA (3- <i>tert</i> -butyl-4-hydroxyanisole), in DMSO, was not genotoxic at concentrations up to 1250 µg/plate to <i>Escherichia coli</i> WP2 strain, with or without metabolic activation.	no

NOTABLE NEW DATA			
Publication	Study Type	Results – Brief Overview	Different from Existing Data?
<b>Other Relevant</b>			
Ham et al. Butylated hydroxyanisole induces testicular dysfunction in mouse testis cells by dysregulating calcium homeostasis and stimulating endoplasmic reticulum stress. <i>Sci Tot Environ.</i> 2020 Feb 1; 702: 134775.	Endocrine activity, in vitro	Immature mouse Leydig cells and immature mouse Sertoli cells were grown in serum-free medium to 60% confluence prior to being treated with 0, 10, 20, 50, 75, or 100 $\mu$ M BHA for 24 h. 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 cells. The relative mRNA expression of genes involved in the biosynthesis of steroids were significantly downregulated after treatment with BHA in both cell lines.	Yes; such data not in original report
Li et al. Effects of butylated hydroxyanisole on the steroidogenesis of rat immature Leydig cells. <i>Tox Mech Meth</i> 2016; 26 (7): 511-519.	Endocrine activity, in vitro	Immature Leydig cells were isolated from 35-d-old rats and cultured with 50 $\mu$ M BHA for 3 h, in combination with 22R-OH-cholesterol, prenenolene, progesterone, androstenedione, testosterone, or dihydrotestosterone, and the concentrations of 5 $\alpha$ -androstenediol and testosterone in the media were measured. Q-PCR was used to measure the mRNA expression levels of the following genes: <i>Lhcgr</i> , <i>Scarb1</i> , <i>Star</i> , <i>Cyp11a1</i> , <i>Hsd3b1</i> , <i>Cyp17a1</i> , <i>Hsd17b3</i> , <i>Srd5a1</i> and <i>Akr1c14</i> . The testis microsomes were prepared to detect the direct action of BHA on 3 $\beta$ -hydroxysteroid dehydrogenase 1 (HSD3B1), 17 $\alpha$ -hydroxylase (CYP17A1) and 17 $\beta$ -hydroxysteroid dehydrogenase-3 activities. BHA significantly inhibited androgen production, rat testis CYP17A1 and HSD3B1 activity, and expression levels of <i>Hsd17b3</i> and <i>Srd5a1</i> .	Yes; such data not in original report
Pop et al. Evaluation of the possible endocrine disruptive effect of butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate in immature female rats. <i>Farmacia</i> 2013; 61 (1): 51 – 60.	Endocrine activity, oral	Immature (17-21 d) female Wistar rats (10/group) received 300 mg/kg bw BHA, 75 mg/kg bw BHT, or 405 mg/kg bw propyl gallate, in sunflower oil, via gavage for 3 d. A 20 $\mu$ g/kg dose of 17 $\beta$ -estradiol, 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 histopathologic changes in the uterus, with no changes in the cervix and vagina. BHA, BHT, and propyl gallate had the ability to decrease the relative uterine weight; propyl gallate had the most severe effect. BHA did exhibit an increase in endometrial epithelium cell height, which was not statistically significant.	Yes; limited data on these effects in original report
Kang et al. Evaluation of estrogenic and androgenic activity of butylated hydroxyanisole in immature female and castrated rats. <i>Toxicol</i> 2005, 213: 147-156.	Endocrine activity, oral	The androgenic activity of BHA was evaluated in an Hersherberger assay. Forty-two-day-old male Sprague-Dawley rats (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, <i>levator ani plus bulbocavernosus</i> 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.	Yes; such data not in the original report

NOTABLE NEW DATA			
Publication	Study Type	Results – Brief Overview	Different from Existing Data?
Kang et al. Evaluation of estrogenic and androgenic activity of butylated hydroxyanisole in immature female and castrated rats. <i>Toxicol</i> 2005, 213: 147-156	Endocrine activity, subcutaneous	Twenty-day-old, female Sprague-Dawley rats were allocated to each test group (11/group). Pups 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 17β-estradiol (in corn oil), administered within 10 min, for 3 d. Six h 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 17β-estradiol-stimulated increase of relative and absolute uterine and vaginal weight. BHA treatment did not affect uterine epithelial height, either alone, or with 17β-estradiol treatment.	Yes; such data not in the original report
Hung, FM, et al. Butylated hydroxyanisole affects immunomodulation and promotes macrophage phagocytosis in normal BALB/c mice. <i>Mol Med Rep.</i> 2012 Mar; 5(3): 683-7.	Immunomodulatory effects	Male BALB/c mice (10/group) received no treatment (controls), olive oil (vehicle controls), 100 or 200 mg/kg BHA, in olive oil, via gavage, for 3 wk. At the end of the treatment, animals were weighed, blood was drawn, and spleen samples were isolated and weighed individually. BHA promoted the levels of T cells and decreased the level of B cells, but did not significantly affect the levels of monocytes and macrophages. No effect on body, liver, or spleen weight of the mice after oral treatment with BHA was observed.	Yes; no data on in vivo immune effects in the original report
Singh et al. Antioxidant butylated hydroxyanisole inhibits estrogen-induced breast carcinogenesis in female ACI rats. <i>J Biochem Mol Toxicol</i> 2009; 23(3): 202-211.	Anti-carcinogenicity	Female ACI rats (≥ 10/group) were treated with cholesterol (controls), BHA, 17β-estradiol (E <sub>2</sub> ), or a combination of 17β-estradiol and BHA for 7, 15, 120, or 240 d, and mammary tumor development was monitored twice weekly during treatment. Rats in the E <sub>2</sub> and E <sub>2</sub> + BHA groups were implanted subcutaneously with pellets of 3 mg E <sub>2</sub> combined with 17 mg cholesterol. Control and BHA only groups had pellets containing only cholesterol implanted. E <sub>2</sub> and control group animals were fed a purified phytoestrogen-free diet, while E <sub>2</sub> + BHA and the BHA only group 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, and brain, ovary tissues were obtained for histopathological analyses. Total 8-isoprostane F <sub>2α</sub> 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 BHA + E <sub>2</sub> group, which exhibited a 24% incidence after 8 mo of treatment compared to the E <sub>2</sub> 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 <sub>2</sub> or E <sub>2</sub> + BHA-treated animals were observed, with the exception of more invasiveness in E <sub>2</sub> -treated mammary tumors. No evidence of tumor or dysplasia was observed in non-target organs. Mammary tissue from rats treated with E <sub>2</sub> + BHA for 120 and 240 d displayed significantly lower levels of 8-isoprostane F <sub>2α</sub> (suggesting inhibition of E <sub>2</sub> -mediated increases) as well increased activity of other antioxidant enzymes, compared to the E <sub>2</sub> only group.	no





**Table 1. 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 <sup>1</sup>	2002 <sup>2</sup>	2023 <sup>3</sup>	2003 <sup>2</sup>
<b>Totals*</b>	<b>70</b>	<b>1224</b>	<b>0.00000004 – 0.15</b>	<b>0.000004 – 0.2</b>
<b>summarized by likely duration and exposure**</b>				
<b>Duration of Use</b>				
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
<b>Exposure Type**</b>				
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 <sup>a</sup> ; 7 <sup>b</sup>	49; 85 <sup>a</sup> ; 88 <sup>b</sup>	0.00000004 – 0.001	0.0001 – 0.2; 0.02 – 0.06 <sup>a</sup> ; 0.004 – 0.1 <sup>b</sup>
Incidental Inhalation-Powder	1; 7 <sup>b</sup>	13; 88 <sup>b</sup> ; 1 <sup>c</sup>	0.05; 0.00013 – 0.013 <sup>c</sup>	0.0002 – 0.005; 0.004 – 0.1 <sup>b</sup> ; 0.0001 <sup>c</sup>
Dermal Contact	57	903	0.00001 – 0.05	0.000004 – 0.2
Deodorant (underarm)	NR	1 <sup>a</sup>	spray: 0.000051 not spray: 0.00076	0.002 <sup>a</sup>
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
<b>as reported by product category</b>				
<b>Baby Products</b>				
Baby Lotions/Oils/Powders/Creams	NR	1	NR	0.0001
<b>Bath Preparations (diluted for use)</b>				
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
<b>Eye Makeup Preparations</b>				
Eyebrow Pencil	NR	51	0.05	0.0001
Eyeliners	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
<b>Fragrance Preparations</b>				
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
<b>Hair Preparations (non-coloring)</b>				
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
<b>Hair Coloring Preparations</b>				
Other Hair Coloring Preparation	1	1	NR	NR
<b>Makeup Preparations</b>				
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
<b>Manicuring Preparations (Nail)</b>				
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
<b>Oral Hygiene Products</b>				
Dentifrices	NR	NR	0.00045	0.01
<b>Personal Cleanliness Products</b>				
Bath Soaps and Detergents	NR	5	0.0006 – 0.0022	0.000004

**Table 1. 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 <sup>1</sup>	2002 <sup>2</sup>	2023 <sup>3</sup>	2003 <sup>2</sup>
Deodorants (underarm)	NR	1	aerosol: 0.000051 not spray: 0.00076	0.002
Other Personal Cleanliness Products	NR	4	NR	0.002
<b>Shaving Preparations</b>				
Aftershave Lotion	NR	2	NR	0.006
Shaving Cream	NR	10	NR	0.0003
Other Shaving Preparations	NR	NR	NR	0.0003
<b>Skin Care Preparations</b>				
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
<b>Suntan Preparations</b>				
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>)<sup>a</sup> It is possible these products are sprays, but it is not specified whether the reported uses are sprays.<sup>b</sup> 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<sup>c</sup> It is possible these products are powders, but it is not specified whether the reported uses are powders.**REFERENCES**

1. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2023. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients (VCRP). (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2023; received February 2, 2023.)
2. Andersen FA (ed). Annual review of cosmetic ingredient safety assessments-2004/2005. *IJT*. 2006;25 1-89.
3. Personal Care Products Council. 2023. Concentration of Use by FDA Product Category: BHA. (Unpublished data submitted by Personal Care Products Council on February 22, 2023.)

## 2

# Final Report on the Safety Assessment of Butylated Hydroxyanisole

Butylated hydroxyanisole (BHA) is used in cosmetic formulations as a chemical preservative and as an antioxidant. 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. Reported acute oral LD<sub>50</sub> values for BHA in rats varied from 2.0 to >5.0 g/kg. Formulations containing BHA elicited, at most, minimal or moderate skin and eye irritation in rabbits. An extensive number of subchronic and chronic oral studies have been conducted and are reviewed.

BHA given orally or parenterally to mice and rats was shown to inhibit the carcinogenic effects of a broad range of chemical carcinogens. BHA has been shown to inhibit mutagenesis and was not a mutagenic agent in standard in vitro tests. No evidence of carcinogenicity was observed when BHA was administered to mice by subcutaneous injection, by intraperitoneal injection, or by topical application. No carcinogenesis was demonstrated following dietary administration of BHA to either rats or dogs. An increased incidence of forestomach papillomas and squamous cell carcinomas has been observed in rats fed BHA. Studies with pregnant rabbits, mice, rats, and hamsters receiving BHA during gestation by a variety of oral dosage regimens revealed no significant embryotoxic or teratogenic effects.

Clinical data for BHA in cosmetic formulations indicated that they were generally nonsensitizing, nonphotosensitizing, and only minimally or mildly irritating. It is concluded that BHA is safe as a cosmetic ingredient in the present practices of use.

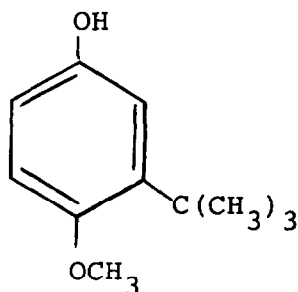
## CHEMICAL AND PHYSICAL PROPERTIES

### Definition and Structure

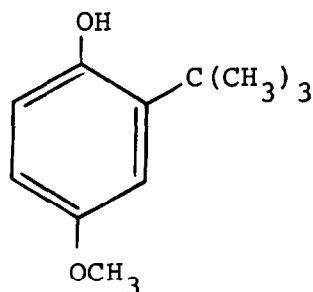
**B**utylated Hydroxyanisole (BHA) (CAS No. 25013-16-5) is a mixture of isomers of tertiary butyl-substituted 4-methoxyphenols.\* Cosmetic grade BHA con-

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\* Additional information on BHA may be found in other literature reviews.<sup>(3,7,10-16)</sup>



3-BHA: approximately 90 percent



2-BHA: approximately 8 percent

sists chiefly of 3-*t*-butyl-4-hydroxyanisole (3-BHA) with lesser amounts of 2-*t*-butyl-4-hydroxyanisole (2-BHA).<sup>(1-3)</sup>

The commercial material by definition contains not less than 98.5 percent of the empirical formula  $C_{11}H_{16}O_2$ .<sup>(2,4)</sup>

### Properties

BHA (mol wt 180.2) is a white or slightly yellow, waxy solid having an aromatic odor. It is insoluble in water but is freely soluble in alcohol, propylene glycol, chloroform, and ether. BHA is slightly soluble in fats and oils.<sup>(5,6)</sup> Cosmetic grade BHA (3-BHA: 90 percent minimum; 2-BHA, approximately 8 percent) is reported to have a melting point of 57°C and a boiling point of 269, 200, 180, 147, and 132°C at 745, 100, 50, 10, and 5 mm Hg, respectively.<sup>(2)</sup> When the isomer mixture consists of approximately 15 percent 2-BHA and 85 percent 3-BHA, the boiling point is between 264 and 270°C at 733 mm Hg, and the melting point between 54 and 58°C.<sup>(3,7)</sup> A melting point range of 48 to 63°C has also been reported.<sup>(6)</sup> BHA is thermostable up to temperatures of 200°C.<sup>(8,9)</sup>

BHA exhibits antioxidant properties and acts synergistically with acids, butylated hydroxytoluene (BHT), propyl gallate, hydroquinone, methionine, lecithin, and thioldipropionic acid in protecting lipids against autooxidation.<sup>(17,18)</sup> The use of a synergistic combination will result in a greater stability than can be obtained by using the equivalent quantity of either antioxidant alone.<sup>(19)</sup>

A BHA sample of > 99 percent 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.<sup>(20)</sup>

The IR spectrum of BHA has been published by Estrin.<sup>(21)</sup>

### Reactivity

BHA undergoes degradation following exposure to sunlight. When the antioxidant was dissolved in soybean oil or lard at either 0.5 or 5.0 percent concentration and subsequently exposed to sunlight for 40 to 70 days, photolytic products consisted of 3,3'-di-*t*-butyl-2,2' dihydroxy-5,5'-dimethoxydiphenyl (A) and 2',3-di-*t*-butyl-2-hydroxy-4', 5-dimethoxydiphenyl ether (B). At a BHA concentration of 5 percent, compound A was formed more than B; at a BHA concentration of 0.5 percent, B was formed more than A.<sup>(22)</sup> Kurechi and Senda<sup>(23)</sup> have also studied the photodegradation of BHA.

Degradation products following a 24-hour exposure of BHA to UV radiation included 2-tert-butyl-quinone; 2-tert-butyl-hydroquinone; 2-tert-butyl-1,4-dimethoxybenzene; 2',3-di-tert-butyl-2-hydroxy-4',5-dimethoxydiphenyl ether; 3,3'-di-tert-butyl-2,2'-dihydroxy-5,5'-dimethoxy biphenyl; 3,3'-di-tert-butyl-2'-hydroxy-2,5,5'-trimethoxy biphenyl; and 3,3'-di-tert-butyl-2,2', 5,5'-tetramethoxy-biphenyl.<sup>(24)</sup>

A benzene solution containing equal molarity BHA and BHT was irradiated with UV radiation. The resulting oxide was confirmed to be 3,3',5'-tri-tert-butyl-5-methoxy-2,4'-dihydroxydiphenylmethane, which is a dehydrogenated dimer of BHA and BHT.<sup>(25)</sup>

The transformation of BHA into the nitrophenol by nitrite under mild acidic conditions prevented the formation of N-nitrosodimethylamine in the reaction between dimethylamine and nitrite. Although the nitrophenol induces no mutagenicity, it was suggested that it might be metabolically transformed into toxic substances, such as hydroxylamine derivatives. The reaction between BHA and nitrite has been shown to yield eight compounds, including 1-hydroxy-2-tert-butyl-4-methoxy-6-nitrobenzene as the major product.<sup>(26)</sup>

BHA is reported to prevent the oxidation of such materials as vitamin D<sub>3</sub>,<sup>(27)</sup> lanolin,<sup>(28)</sup> vitamin A,<sup>(29)</sup> vitamin A esters,<sup>(30)</sup> methyl oleate,<sup>(31)</sup> cod-liver oil,<sup>(32)</sup> and various fatty bases.<sup>(33)</sup> BHA reduces rancidity and improves stability by providing reactive hydrogen atoms to lipid-free radicals.<sup>(34,35)</sup> The resulting antioxidant free radical usually has several stable resonance forms, which in turn prevent further stimulation of free radical formation (Fig. 1).<sup>(36)</sup> Thus, BHA prevents lipid peroxidation by acting as a chain-breaker in autooxidation processes.<sup>(37)</sup> Theories relating to antioxidant properties of BHA in cosmetic formulations and raw materials have been reviewed by Marcinkiewicz.<sup>(38)</sup>

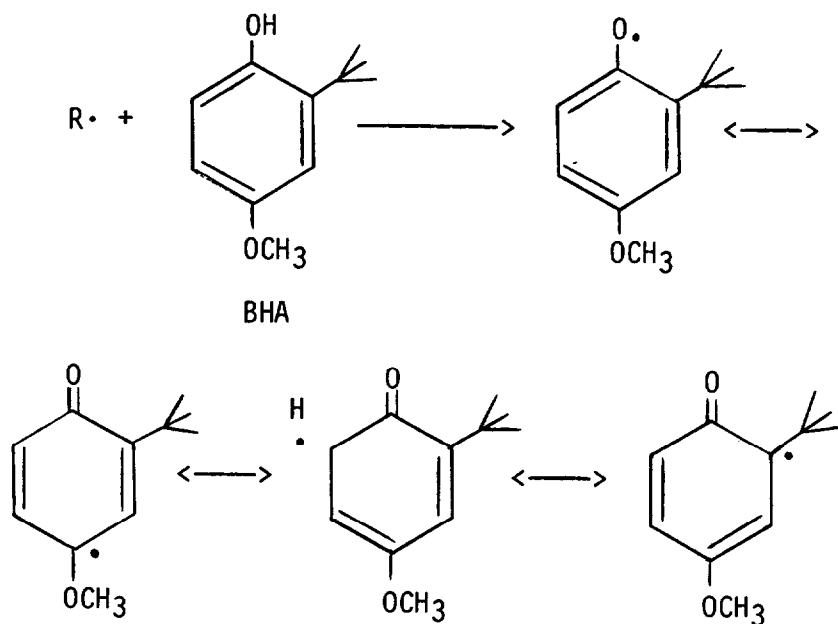


Figure 1. Stable resonance forms of BHA.<sup>(36)</sup>

Studies reporting evidence of interactions between vitamin E and BHA have been reviewed by the Select Committee on GRAS Substances.<sup>(11)</sup> However, the nature of these interactions remains to be established. BHA is also known to react readily with oxidizing agents to give quinones.<sup>(2)</sup>

BHA is often referred to as a "hindered phenol" because the reactivity of the phenol is decreased by the tert-butyl substitution in the ortho position.<sup>(37)</sup>

### Analytical Methods

Analytical methods for the determination of BHA include gas and gas-liquid chromatography,<sup>(39-45)</sup> paper chromatography,<sup>(46)</sup> thin-layer chromatography,<sup>(47-53)</sup> colorimetric techniques,<sup>(54-56)</sup> fluorometric techniques,<sup>(57,58)</sup> nuclear magnetic resonance,<sup>(3)</sup> voltammetric techniques,<sup>(59)</sup> gel permeation chromatography,<sup>(60)</sup> liquid and/or high performance liquid chromatography<sup>(61-67)</sup> polarographic techniques,<sup>(68)</sup> lipophilic gel chromatography,<sup>(69)</sup> spectrophotometry,<sup>(70-73)</sup> and densitometric techniques.<sup>(56,74)</sup> A sensitive method for the detection of picomole amounts of BHA in aqueous samples has also been reported.<sup>(75)</sup>

Several literature reviews relating to the analytical determination of BHA have been published.<sup>(3,76,77)</sup>

### Method of Manufacture

BHA can be synthesized either by tert-butylation of *p*-methoxyphenol<sup>(78)</sup> or by methylation of tert-butylhydroquinone.<sup>(2,78,79)</sup> BHA can also be prepared from *p*-methoxyphenol and isobutene.<sup>(17)</sup> The product is purified by distillation and subsequently supplied to cosmetic formulators in the form of tablets or flakes.<sup>(2)</sup> Lam et al.<sup>(78,80)</sup> have described methods for the synthesis of both 2-BHA and 3-BHA in their pure isomeric forms.

### Impurities

The following impurities have been reported for BHA as it is used in cosmetics<sup>(2,4)</sup>:

4-Hydroxyanisole	0.5 percent maximum
1-t-Butyl-2,5-Dimethoxybenzene	0.5 percent maximum
2,5-Di-t-Butyl-Hydroxyanisole	0.2 percent maximum
Hydroquinone Dimethyl Ether	0.1 percent maximum
Sulfated Ash	0.01 percent maximum
Lead (as Pb)	20 ppm maximum
Arsenic (as As)	3 ppm maximum

Food-grade BHA is also reported to contain hydroxyanisole and hydroquinone at levels of 0.5 and 0.6 percent (maximum), respectively.<sup>(3)</sup>

## USE

### Purpose in Cosmetics

BHA is used in cosmetic formulations as a chemical preservative<sup>(81)</sup> and as an antioxidant.<sup>(2,82)</sup>

### Scope and Extent of Use in Cosmetics

Data submitted to the Food and Drug Administration (FDA) in 1981 by cosmetic firms participating in the voluntary cosmetic registration program indicated that BHA was used as an ingredient in a total of 3217 cosmetic formulations at concentrations of > 10 to 25 percent (1 product), > 1 to 5 percent (3 products), >0.1 to 1 percent (217 products), and  $\leq$ 0.1 percent (1693 products) (Table 1). The BHA concentration was not reported for 1303 products. The greatest reported use of the antioxidant was in eye shadow (410 products) and lipstick (1256 products).<sup>(83)</sup>

Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators conforms to the prescribed format of preset concentration ranges and product categories as described in Title 21 Part 720.4 of the Code of Federal Regulations (21 CFR 720.4). Because data are only submitted within the framework of preset concentration ranges, opportunity exists for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to tenfold error in the assumed ingredient concentration.

### Surfaces to Which Applied

Cosmetic products containing BHA are applied to or have the potential to come in contact with skin, eyes, hair, nails, and vaginal and nasal mucosa. Small amounts of the ingredient could be ingested from lipstick (Table 1).

### Frequency and Duration of Application

Product formulations containing BHA may be used from once a week to several times a day. Many of the products may be expected to remain in contact with body surfaces for as briefly as a few minutes to as long as a few days. Each product has the potential for being applied hundreds of times over the course of several years (Table 1).

### Noncosmetic Use

BHA is used as an antioxidant in edible fats and oils, fat-containing foods, waxes, essential oils, food-coating materials, and vitamin A preparations.<sup>(6,11,17,19,34,35,84-86)</sup> The compound is classified as a Generally Recognized As Safe (GRAS) food preservative and may be used as such at concentrations not to exceed 0.02 percent (w/w) of the total fat or oil content of a particular food. If BHA is used in foods in combination with other antioxidants, the total antioxidant content may not exceed 0.02 percent w/w (21 CFR 182.3169). Regulations of the US Department of Agriculture place limits of not more than 0.01 percent of total product weight for BHA in certain meat products (9 CFR Part 318.7 [c][4]). In addition to use as a GRAS substance, BHA may be used under the Federal Food, Drug and Cosmetic Act as an antioxidant in specific foods at the following maximum levels (21 CFR 172.110; 172.515; 172.615):

TABLE 1. Product Formulation Data.<sup>(83)</sup>

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (%)*					
			Unreported Concentration	>10-25	>5-10	>1-5	>0.1-1	≤0.1
<i>BHA</i>								
Baby lotions, oils, powders, and creams	35	1	—	—	—	—	1	—
Bath oils, tablets, and salts	237	20	9	—	—	—	—	11
Bubble baths	475	7	2	—	—	—	—	5
Other bath preparations	132	10	5	—	—	—	1	4
Eyebrow pencil	145	33	1	—	—	—	2	30
Eyeliners	369	75	8	—	—	—	3	64
Eye shadow	2582	410	105	—	—	1	66	238
Eye lotion	13	2	1	—	—	—	—	1
Eye makeup remover	81	11	1	—	—	—	—	10
Mascara	397	65	13	—	—	—	2	50
Other eye makeup preparations	230	39	12	—	—	—	1	26
Colognes and toilet waters	1120	97	1	—	—	—	10	86
Perfumes	657	62	1	—	—	—	26	35
Fragrance powders (dusting and talcum, excluding aftershave talc)	483	12	4	—	—	—	—	8
Sachets	119	21	1	—	—	—	—	20
Other fragrance preparations	191	24	3	—	—	—	3	18
Hair conditioners	478	8	5	—	—	—	—	3
Hair sprays (aerosol fixatives)	265	1	1	—	—	—	—	—
Hair shampoos (noncoloring)	909	6	1	—	—	—	—	5
Tonics, dressings, and other hair grooming aids	290	10	3	—	—	—	2	5
Wave sets	180	1	1	—	—	—	—	—
Other hair coloring preparations	49	5	—	—	—	—	—	5



Blushers (all types)	819	176	56	—	—	1	9	110
Face powders	555	98	28	—	—	—	7	63
Makeup foundations	740	119	37	—	—	—	—	82
Lipstick	3319	1256	773	1	—	—	37	445
Makeup bases	831	64	51	—	—	—	1	12
Rouges	211	48	21	—	—	—	1	26
Makeup fixatives	22	10	—	—	—	—	—	10
Other makeup preparations (not eye)	530	106	52	—	—	1	14	39
Nail basecoats and undercoats	44	1	—	—	—	—	—	1
Cuticle softeners	32	2	—	—	—	—	—	2
Nail creams and lotions	25	4	3	—	—	—	—	1
Nail polish and enamel remover	41	1	—	—	—	—	—	1
Other manicuring preparations	50	2	—	—	—	—	—	2
Bath soaps and detergents	148	2	—	—	—	—	—	2
Deodorants (underarm)	239	1	—	—	—	—	—	1
Other personal cleanliness products	227	2	—	—	—	—	1	1
Aftershave lotions	282	11	1	—	—	—	1	9
Preshave lotions (all types)	29	3	3	—	—	—	—	—
Shaving cream (aerosol) brushless and lather	114	8	—	—	—	—	—	8
Shaving soap (cakes, sticks, and so on)	7	1	—	—	—	—	1	—
Other shaving preparation products	29	3	—	—	—	—	2	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	680	51	11	—	—	—	3	37
Face, body, and hand skin care preparations (excluding shaving preparations)	823	77	15	—	—	—	11	51
Hormone skin care preparations	10	1	1	—	—	—	—	—

TABLE 1. (Continued.)

Product Category*	Total No. of Formulations in Category	Total No. Containing Ingredient	No. of Product Formulations Within Each Concentration Range (%)*					
			Unreported Concentration	>10-25	>5-10	>1-5	>0.1-1	≤0.1
<i>BHA con't</i>								
Moisturizing skin care preparations	747	111	29	—	—	—	4	78
Night skin care preparations	219	30	7	—	—	—	3	20
Paste masks (mud packs)	171	6	1	—	—	—	2	3
Skin lighteners	44	11	4	—	—	—	—	7
Skin fresheners	260	6	2	—	—	—	—	4
Wrinkle smoothers (removers)	38	6	3	—	—	—	—	3
Other skin care preparations	349	42	5	—	—	—	1	36
Suntan gels, creams, and liquids	164	27	19	—	—	—	2	6
Indoor tanning preparations	15	2	—	—	—	—	—	2
Other suntan preparations	28	9	3	—	—	—	—	6
1981 TOTALS		3217	1303	1	—	3	217	1693

\*Preset product categories and concentration ranges in accordance with federal filing regulations (21 CFR 720.4).

<i>Food</i>	<i>Max. Level of BHA Permitted</i>
Potato granules	10 ppm, alone or with BHT
Mixed, diced, glazed, fruits	32 ppm
Dry breakfast cereals, sweet potato flakes, dehydrated potato flakes or shreds	50 ppm, alone or with BHT
Dry mixes for beverages and desserts	90 ppm in mix or <2 ppm in prepared foods
Emulsion stabilizers for shortenings	200 ppm, alone or with BHT
Active dry yeast	0.1 percent
Chewing gum	0.1 percent, alone or with BHT and/or propyl gallate
Flavoring substances	Not to exceed 0.5 percent of the essential oil content of the flavoring substance

The Joint FAO/WHO Expert Committee on Food Additives<sup>(16)</sup> suggested that a dietary level not exceeding 0.5 mg/kg body weight of BHA, BHT, or the sum of both would be an acceptable daily intake for man. The daily dietary intake of man is estimated to be 0.05 to 3 mg.<sup>(11)</sup>

BHA may be used as an antioxidant in defoaming agents for the processing of beet sugar and yeast at levels not to exceed 0.1 percent of the defoamer (21 CFR 173.340). It may also be added to food packaging materials at levels not to exceed 0.005 percent (21 CFR 181.24) and to both lubricants (21 CFR 178.3570) and adhesives (21 CFR 175.105) in contact with food. No specific limitations for use of BHA in lubricants or adhesives have been established.

An FDA Advisory Review Panel on drug products for over-the-counter human use has classified BHA as an "inactive ingredient or pharmaceutical necessity" in external analgesics. When used in concentrations at the level of or above the minimum effective dose (this dose was not reported), it is considered an active ingredient.<sup>(87)</sup>

## BIOLOGICAL PROPERTIES

### Antimicrobial Properties

BHA inhibits the growth of a wide variety of microorganisms. This inhibition is both species- and dose-dependent.<sup>(88)</sup> It has been suggested that disruption of the cytoplasmic membrane is at least partially responsible for the compound's inhibitory power.<sup>(36,89)</sup>

The minimum BHA concentration required to inhibit the growth of various microorganisms in liquid culture medium (pH 7.0) has been reported by Kabara<sup>(90)</sup> (Table 2).

BHA concentrations of 25 to 400 ppm have been found to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Vibrio parahaemolyticus*, *Clostridium perfringens*, *Clostridium botulinum*, *Salmonella typhimurium*, and *Pseudomonas fluorescens*.<sup>(36,91-101)</sup>

BHA shows a synergistic inhibitory effect against *C. perfringens* when used in

**TABLE 2.** Minimum Inhibitory Concentration of BHA in Liquid Culture Medium (pH 7.0).<sup>(90)</sup>

Microorganism	BHA
<i>Escherichia coli</i>	2000 $\mu\text{g/ml}$
<i>Pseudomonas aeruginosa</i>	> 5000 $\mu\text{g/ml}$
<i>Streptococcus mutans</i>	125 $\mu\text{g/ml}$
<i>Streptococcus agalactiae</i>	125 $\mu\text{g/ml}$
<i>Staphylococcus aureus</i>	250 $\mu\text{g/ml}$
<i>Corynebacterium sp.</i>	125 $\mu\text{g/ml}$
<i>Nocardia asteroides</i>	250 $\mu\text{g/ml}$
<i>Saccharomyces cerevisiae</i>	125 $\mu\text{g/ml}$
<i>Candida albicans</i>	250 $\mu\text{g/ml}$

conjunction with nitrite, sorbic acid, or parabens; however, in the presence of lipid or surfactant, the antimicrobial activity of BHA is greatly reduced.<sup>(95)</sup> Beggs et al.<sup>(102)</sup> reported synergistic antimicrobial activity of amphotericin B, an antifungal antibiotic, against the yeasts *Candida albicans* and *Candida parapsilosis* in the presence of subinhibitory concentrations of BHA, (0.6  $\mu\text{g/ml}$ ). Potassium sorbate at a concentration of 0.2 percent (w/w) in growth media along with 100 ppm BHA also acts synergistically to inhibit *S. aureus*.<sup>(103)</sup> BHA is more effective as an antimicrobial agent against *S. aureus* as the pH of the growth medium is lowered and the NaCl concentration of the medium is increased.<sup>(93)</sup>

BHA (20 ppm) inhibited both cell growth and synthesis of DNA, RNA, and protein in *Tetrahymena pyriformis*. Protozoa exposed to this level had a normal morphology and size distribution.<sup>(104)</sup> The inhibitory effect of BHA on *Tetrahymena* might be caused by binding of the phenolic compound to protein or nucleoprotein.<sup>(18)</sup>

BHA at levels of 0.005 to 0.02 g per plate of solid medium<sup>(105)</sup> and 100 ppm<sup>(106)</sup> inhibited the growth of *Aspergillus flavus*, whereas 250 ppm was fungicidal to *Aspergillus parasiticus*.<sup>(99)</sup> Ahmad<sup>(107)</sup> reported that 200 ppm of BHA in growth medium caused 100 percent inhibition of *Geotrichum*, *Penicillium*, and *Aspergillus*. A concentration of 2 percent (w/w) BHA in growth medium inhibited both *Trogoderma variable* and *Attagenus megatoma*.<sup>(108)</sup>

BHA (100  $\mu\text{M}$ ) was also reported to be effective in inhibiting the growth of bacteriophage  $\phi$  6.<sup>(109)</sup>

Davidson<sup>(36)</sup> and Branen et al.<sup>(110)</sup> have reviewed the literature about the antimicrobial properties of BHA. These authors discussed the compound's activity against bacteria, molds, viruses, and protozoa, the effect of pH, temperature, and the presence of lipids on the effectiveness of BHA's antimicrobial properties, and the possible mechanism(s) of microbial inhibition.

### Effect on Melanocytes

Riley<sup>(111)</sup> found that BHA concentrations of  $5 \times 10^{-3}\text{M}$  were toxic to cultured guinea pig melanocytes, whereas BHA at  $5 \times 10^{-6}\text{M}$  caused no melanocyte damage. The morphologic damage to guinea pig melanocytes exposed in vitro simultaneously to *p*-hydroxyanisole and BHA was also assessed by Riley et al.<sup>(112)</sup> BHA at concentrations of  $10^{-2}\text{M}$  reduced cytotoxicity to melanocytes caused by

*p*-hydroxyanisole. However, BHA acted synergistically with *p*-hydroxyanisole to increase cellular damage as the BHA concentration decreased from  $10^{-2}M$  to  $10^{-6}M$ .

BHA at concentrations of 0.1 to 1.0 M in various solvents failed to induce depigmentation when applied to the skin of "two to five" guinea pigs each weekday for "1-6" months. No depigmentation was observed when similar concentrations of BHA were applied each weekday to the skin of 10 black mice for 2 to 4 months.<sup>(113)</sup>

### Effect on Behavior

Pregnant mice received a diet supplemented with 0.5 percent (by weight) BHA throughout gestation. The newborn offspring at 30 days of age showed reductions in exploratory reflex, body weight, and brain cholinesterase activity.<sup>(114)</sup>

Stokes and Scudder<sup>(115)</sup> observed altered behavior patterns in Swiss-Webster mice fed diets containing 0.5 percent (w/w) BHA. Dams received the BHA ration during pregnancy and weaning; pups were fed the diet during weaning and early growth (up to 6 or 7 weeks of age). No significant differences were noted between treated and control animals with respect to digging, freezing behavior, carrying, grooming or contactual, and sexual behavior. However, treated animals did show increased nest exploration behavior and decreased sleeping, self-grooming, learning rates, and orientation reflexes.

In studies by Barcus,<sup>(116)</sup> no evidence was obtained to support the hypothesis that hyperactivity in dogs is related to the presence of BHA in the diet. The compound was given at 1.91 ppm for 18 days in the food of telomian hybrid beagle dogs, a breed purported to exhibit behavior analogous to hyperactivity in children. However, the author acknowledged a number of deficiencies in test methodology, including lack of a control group and a small number of animals tested (four).

### Effect on Hormones

Boehme and Branen<sup>(117)</sup> investigated the effects of various antioxidants on prostaglandin biosynthesis by microsomal fractions from bovine seminal vesicles. Concentrations of 6.70 and 3.08  $\mu M$  BHA produced a 50 percent inhibition of prostaglandin  $E_1$  ( $PGE_1$ ) and prostaglandin  $E_2$  ( $PGE_2$ ), respectively. In vitro, 1.06  $\mu M$  of BHA inhibited  $PGE_2$  biosynthesis by 28 percent and stimulated  $PGE_1$  biosynthesis by 34 percent. For a human adult weighting 64 kg and consuming 0.1 mg/kg/day of food antioxidant, the authors calculated that the whole body concentration for a single antioxidant/day could be as high as 1.06  $\mu M$  for BHA, 0.7  $\mu M$  for BHT, 1.1  $\mu M$  for ascorbic acid, and 1.15  $\mu M$  for t-butyl hydroquinone (TBHQ). On the basis of these estimations, they suggested that BHA could have a profound effect on prostaglandin biosynthesis in vivo. However, it was noted that these antioxidant levels might never be reached in the glands responsible for prostaglandin synthesis.

Zenser and Davis<sup>(118)</sup> observed a concentration-dependent inhibition of prostaglandin production in slices of rat renal medulla treated with BHA. Prostaglandin production was inhibited at 1 mM BHA but not at 0.01 or 0.1 mM. The antioxidant also blocked arginine vasopressin-mediated increases in cAMP at a level of 1mM. Direct inhibition of medullary adenylate cyclase or a toxic effect are possible explanations for these phenomena.

Egan et al.<sup>(119)</sup> reported that BHA stimulated prostaglandin biosynthesis (as indicated by cyclooxygenase activity) at concentrations of 30  $\mu\text{M}$  but inhibited biosynthesis at 160  $\mu\text{M}$ .

Volkova<sup>(120)</sup> observed no alteration of pituitary gonadotrophic hormone in albino rats or guinea pigs fed BHA at a dose of 0.4 mg/kg/day for 6 months.

Inhibition of bradykinin activity was noted in isolated rat uterine horn muscle treated with  $10^{-6}\text{M}$  BHA. The antioxidant interacted directly with the polypeptide kinin to impair the latter's function.<sup>(121)</sup> Posati and Pallanch<sup>(122)</sup> described the competitive inhibition of bradykinin by BHA in isolated smooth muscle from guinea pig ileum. Antioxidant concentrations as low as  $8 \times 10^{-9}$  mole/L suppressed the contractile response of smooth muscle to bradykinin.

### Anticarcinogenic Effects

BHA administered orally or parenterally to mice and rats has been shown to inhibit the effects of a broad range of chemical carcinogens under a variety of experimental conditions.<sup>(123)</sup> Suppression of neoplasms by BHA has been demonstrated in experiments in which the route of carcinogen administration results in direct contact of carcinogen with the target tissue (as in neoplasia of the forestomach in mice fed benzo[a]pyrene or 7,12-dimethylbenz[a]anthracene), as well as in experiments in which the carcinogen is acting at a site remote from that of carcinogen administration (as in mammary tumor formation in rats given 7,12-dimethylbenz[a]anthracene orally).<sup>(124,125)</sup> The BHA-mediated protection of rodents against the neoplastic effects of carcinogens is considered by Benson et al.<sup>(126)</sup> to be nonspecific with respect to the chemical nature of the carcinogen, the route of carcinogen administration, or the site of tumor formation.

The mechanism of the anticarcinogenic effect of BHA has not been elucidated. Potential mechanisms include (1) alteration of 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.<sup>(127,128)</sup> With regard to the last mechanism, Awasthi et al.<sup>(129)</sup> have 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.

In studies by Sлага and Braken,<sup>(130)</sup> 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 authors suggested that BHA has an indirect effect on the epidermal metabolizing system, which leads to a decrease in covalent binding of carcinogen to DNA. It was also suggested that inhibition of polycyclic hydrocarbon tumorigenesis by BHA may be related either to (1) the ability of the antioxidant to prevent the *in vivo* activation of hydrocarbons to carcinogenic epoxides and/or other electrophilic intermediates or (2) the ability of BHA to increase detoxification of the reactive intermediates.

Rahimtula et al.<sup>(131)</sup> reported that BHA (100  $\mu\text{M}$ ) can inhibit cytochrome P-450 and other hemoprotein-catalyzed oxidation of drugs and carcinogens. They suggested three possible mechanisms of antioxidant inhibition: (1) as an inhibitor of drug hydroxylation, (2) as a peroxidase donor serving to discharge the active hydroxylating complex, and (3) as a free radical scavenger.

Other studies have shown that BHA exhibits pronounced effects on enzymes involved directly or indirectly in the metabolism of carcinogens.<sup>(126)</sup> Benson et al.<sup>(126,132,133)</sup> demonstrated that addition of BHA to the diet of mice and rats significantly raises the levels of hepatic and extrahepatic glutathione S-transferases. These enzymes purportedly act to detoxify and thereby inhibit the metabolites of procarcinogens by catalyzing the latter's conjugation with glutathione.<sup>(126,129)</sup> Dietary BHA has also been shown to induce hepatic and extrahepatic epoxide hydratase, an enzyme that functions in both the metabolic activation and inactivation of the carcinogen benzo[a]pyrene.<sup>(132,134)</sup> Thus, the ability of BHA to elevate glutathione S-transferase and epoxide hydratase activities suggests that increased enzymatic inactivation of carcinogens or of reactive metabolites may be involved in the mechanism of protection of this antioxidant.<sup>(132)</sup>

BHA was not effective in inhibiting 7,12-dimethylbenz[a]anthracene-induced mammary tumors when rats were fed diets containing 0.7 percent of the antioxidant and either 20 percent corn oil, 18 percent coconut oil plus 2 percent linoleic acid, or 2 percent linoleic acid. Results suggested that the effectiveness of BHA as a tumor inhibitor may be altered by dietary factor.<sup>(135,136)</sup>

Numerous other papers documenting the anticarcinogenic activity of BHA and its isomers have been published.<sup>(78,80,137-175)</sup>

### **Antimutagenic Effects**

In addition to inhibition of carcinogenesis, BHA has been found to inhibit mutagenesis, both in vivo and in vitro.<sup>(176-183)</sup> 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.<sup>(126,184)</sup> Dolara et al.<sup>(185)</sup> suggest that the antimutagenic effect of dietary BHA may have important exceptions. They observed a higher activation of beef-extract mutagens in CD-1 mice following the addition of 0.75 percent BHA to the animals' diet.

### **Effect on Enzymatic Activity**

The ability of BHA to affect the activity of a number of enzymes is well documented. However, whether the antioxidant inhibits or stimulates enzyme activities varies according to test conditions (Table 3). Quantitative changes in enzymatic activity due to BHA treatment appear to be related to dosage and duration of administration.<sup>(11)</sup>

Induction of drug-metabolizing enzymes by BHA is often accompanied by liver enlargement. The Select Committee on GRAS Substances<sup>(11)</sup> 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, they suggested that liver enlargement (variously termed "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.

### **Effect on Acid-Soluble Thiol Compounds in Tissues of Mice**

Nonprotein, acid-soluble thiols in the jejunal and duodenal mucosa of CD-1 mice fed a diet containing 0.75 percent BHA for 10 days were elevated to 238

**TABLE 3.** Effect of BHA on Enzymatic Activity.

<i>BHA Concentration</i>	<i>Test System</i>	<i>Enzyme(s)</i>	<i>Effect</i>	<i>Other Observations</i>	<i>Ref.</i>
50 and 200 $\mu$ M	Isolated rat liver microsome	Benzpyrene hydroxylase <i>p</i> -Nitroanisole demethylase Ethylmorphine demethylase Aminopyrine demethylase Benzphetamine demethylase	Inhibited Inhibited Inhibited Inhibited Inhibited	BHA became bound to cytochrome P-450 but did not inhibit reduced NADPH-cytochrome c reductase activity	186
4 $\mu$ g	Isolated rat liver microsome	NADPH-lipid peroxidase system	Inhibited		186
500 mg/kg/day for 21 days by stomach tube	Rat liver microsome	Biphenyl-4-hydroxylase BHT-oxidase UDPG-dehydrogenase	Transient activation Transient activation Activated	Rats showed increased liver weights within 24 hours of initial dose; this increase followed a bimodal time course; increased urinary excretion of ascorbic acid associated with the first maximum	187
0.5 percent of diet	Rat liver microsome	Biphenyl-4-hydroxylase BHT-oxidase	Transient activation Transient activation	Increase in activity of these two mixed function oxidases (Day 1-3 following BHA, administration) not accompanied by liver enlargement did not occur until Day 5	187
Oral doses of 1.5 mmoles/kg/day for 6 days	Rat liver	Hexobarbitone oxidase Aminopyrine demethylase	No observed induction No observed induction	Increase in liver size observed; concluded that liver enlargement not invariably related to induction of drug-metabolizing activities	20,188
0.2 mmol/L	Microsomal cell fraction of rat liver	Enzymatically induced lipid peroxidation Decrease in monooxygenase caused by peroxidation of membrane lipids Increase in UDP glucuronosyltransferase ( <i>p</i> -nitrophenol) caused by peroxidation of membrane lipids	Inhibited Inhibited Inhibited		189



10 mmol/L	Microsomal cell fraction of rat liver	Monoxygenase	Inhibited		189
		UDP glucuronosyltransferase	Inhibited		
100 mg/kg in diet	Rat blood	Sugar cholinesterase	Inhibited	Significant decreases in ketone bodies observed	190
600 mg/kg in diet	Rat blood	Catalase	Inhibited		191,192
		Peroxidase	Inhibited		
500 mg/kg/day for 4 or 7 days by oral intubation	Rat liver	Glucose-6-phosphatase	No change		193
		Glucose-6-phosphate dehydrogenase	No change		
0.1 percent of diet for 16 weeks	Rat liver	Glucose-6-phosphatase	No change activated after 4 weeks in females		194
		Glucose-6-phosphate dehydrogenase			
500 mg/kg/day for 84 days by stomach intubation	Rat liver	Nitroanisole demethylase	No change	Increase in protein content and weight of the liver observed, elevated liver weight and urinary ascorbate also occurred in rats given 500 mg/kg BHA in diet, but these increases returned to normal by 6th day of treatment	195,196
		Aminopyrine demethylase	No change		
		Hexobarbitone oxidase	No change		
		Codeine demethylase	No change		
0.1 percent (1000 ppm) or 0.25 percent (2500 ppm) of diet for 12 days	Rat liver microsome	Biphenyl-4-hydroxylase	No effect	No effect on liver weight	197
		4-methoxybiphenyl demethylase	No effect		
0.5 percent of diet for 9 days	Rat liver microsome	Biphenyl-4-hydroxylase	Activated	No effect on liver weight	197
500 mg/day/os for 3 days	Rat liver	Benzo[a]pyrene hydroxylase	No change	No increase in liver size	198
0.75 percent of diet for 10 days	Mouse liver microsome	Epoxide hydratase	Activated		199
750 mg/kg/day for 4 days by gastric intubation	Mouse liver microsome	Epoxide hydratase	Activated		199
0.75 percent of diet for 3 or 12 days	Mouse liver microsome	Benzo[a]pyrene hydroxylase	No effect	No change in cytochrome P-450 content	199

TABLE 3. (Continued.)

BHA Concentration	Test System	Enzyme(s)	Effect	Other Observations	Ref.
0.75 percent of diet for 10 days	Rat liver microsome	Aminopyrine demethylase	No effect		
		Epoxide hydratase	Activated		
		Epoxide hydratase	Activated		199
0.75 percent of diet for 8 days	Microsomal and cytosol fractions of rat and mouse liver	Epoxide hydratase	Activated	Increase in cytochrome b <sub>5</sub> and decrease in cytochrome P-450 content observed	200
		Aniline hydroxylase	Activated		
		UDP-glucuronic acid transferase	Activated		
		NADPH cytochrome c reductase	Activated		
		NADH cytochrome c reductase	Activated		
		Glucose-6-P-dehydrogenase	Activated		
		UDPG dehydrogenase	Activated		
0.1 or 0.5 percent of diet for 14 days	Rat liver microsome	Benzo[a]pyrene hydroxylase	Inhibited	Cytochrome b <sub>5</sub> concentrations were elevated whereas cytochrome P-450 concentrations remained similar to control values	201,202
		Aminopyrine demethylase	Inhibited		
		Glucose-6-phosphatase	Inhibited		
		Epoxide hydratase	Activated		
		Ethoxycoumarin deethylase	Activated		
50 $\mu$ M	Phenobarbital-stimulated rat liver microsomes	Arylhydrocarbon hydroxylase	Inhibited		201,202
Up to 500 $\mu$ M	3-methylcholanthrene-stimulated rat liver microsomes	Arylhydrocarbon hydroxylase	No effect		201,202
0.75 percent of diet for 14 days	Microsomal fractions of mouse liver, kidney, lung, stomach, small intestine, colon, and thymus	Epoxide hydratase	Activated		132,133

0.75 percent of diet for 8 days	Cytosol fractions of mouse liver, kidney, lung, stomach, small intestine, uterus, thymus, spleen	Glutathione S-transferase	Activated in liver, kidney, lung, stomach, small intestine, and colon, but no change in uterus, thymus, or spleen		
	Microsomal fraction of rat liver, small intestine, kidney, lung, spleen, testis, and brain	Epoxide hydratase	Activated in liver, small intestine, kidney, and lung but no changes in spleen, testis, or brain		132,133
15 mg/day for 10 days in diet	Cytosol fraction of rat liver, small intestine, kidney, lung, spleen, testis, and brain	Glutathione S-transferase	Activated in liver, small intestine, kidney, and lung but no changes in spleen, testis, or brain		
	Microsomal fraction of mouse liver	Aniline hydroxylase	Activated	Decreases observed in cytochrome P-450 content, whereas increases occurred in cytochrome b <sub>5</sub> content and activities of NADPH and NADH-dependent cytochrome c reductases; increased size and protein content of liver also noted	203
		UDP-glucuronyltransferase	Activated		
Glucose-6-phosphatase		Activated			
	Cytosol fraction of mouse liver	Amino-demethylase	Inhibited		
		Benzo[a]pyrene hydroxylase	Inhibited		
		Glucose-6-phosphate dehydrogenase	Activated	Increased protein content	
5 mg/g of chow (75 mg/kg of body weight)	Mouse liver microsomes	UDP-glucose dehydrogenase	Activated		
		Mixed function oxidase system	Altered	Decreases in cytochrome P-450 content and production of epoxides of benzo[a]pyrene occurred in liver extracts. Authors suggested that BHA inhibits carcinogenesis of benzo[a]pyrene by increasing its detoxification and decreasing its activation as a result of the effects on mixed oxidase enzyme systems	145

TABLE 3. (Continued.)

<i>BHA Concentration</i>	<i>Test System</i>	<i>Enzyme(s)</i>	<i>Effect</i>	<i>Other Observations</i>	<i>Ref.</i>
15 $\mu$ M	Liver, lung, and skin of rats and mice	Arylhydrocarbon hydroxylase	Inhibited		204
50 or 100 mg followed 1 hour by intratracheal administration of 1 mg benzo[a]pyrene	Hamster lungs	Arylhydrocarbon hydroxylase	Inhibited		205
$10^{-4}$ to $10^{-2}$ M	Microsomal fraction of rat liver	Arylhydrocarbon hydroxylase	Inhibited		206
	Human placenta	Arylhydrocarbon hydroxylase	No effect		207
	Mouse epidermis	Arylhydrocarbon hydroxylase	No effect		130
0.25 to 3 mM	Liver and/or colon of rats, mice, and rabbits	Guanylate cyclase	Inhibited	The suppression of the carcinogen activation of guanylate cyclase could be involved in the anti-carcinogenic properties of BHA	138-141
Oral doses ranging from 100 to 1000 mg/kg for 4 days	Cytosol fraction of mouse liver	Glutathione-S-transferases	Activated		126

	Cytosol fraction of rat liver	Glutathione-S-transferases	Activated	126
5 mg/g in diet for 13 days	Cytosol fraction of liver and mucosa of small intestine	Glutathione-S-transferases	Activated	208
0.01 to 2.0 micromol/ml	Unpurified liver and large intestine of mice	NAD <sup>+</sup> -dependent alcohol dehydrogenase	Inhibited	172
Single IP injection of 62.5, 215, or 500 mg/kg	Lung of mice	Superoxide dismutase	No change	209
		Glutathione reductase	No change	
		Glutathione peroxidase	No change	
		Glucose-6-phosphatase	No change	
0.5 percent in diet of mother mice for 30 days	Brain of newborn mice	Cholinesterase	Inhibited	114
4 mg/kg/day in diet for 30 to 35 days	Mouse pancreas	Amylase	No effect	192
		Lipase	No effect	
	Mouse intestine	Enterokinase	No effect	
		Alkaline phosphatase	No effect	
Oral doses of 500 mg/kg/day	Monkey liver microsome	Glucose-6-phosphatase	Inhibited	210
		Nitroanisole demethylase	Activated	
			Slight decreases in liver succinic dehydrogenase, blood catalase and serum phosphatase were not significant; cytochrome P-450 content was not altered	

percent of control values. Modest elevations (24 to 32 percent) in acid-soluble thiol compounds were observed in the stomach, ileum, colon, and urinary bladder, whereas concentrations in the heart, spleen, diaphragm, and skeletal muscle were unaffected by dietary BHA. Elevation of thiol compounds in the liver and extrahepatic tissues was further evidence that enhancement of detoxification of carcinogens and their metabolites may constitute part of the biochemical mechanisms by which BHA protects against chemical carcinogenesis.<sup>(132,133)</sup>

### Other in Vitro and in Vivo Effects

BHA has been shown to demonstrate biological activity in a number of in vitro and in vivo test systems. Results of such studies are summarized in Table 4.

### Absorption, Metabolism, Excretion, and Storage

Both animal and human studies have established that BHA is absorbed from the gastrointestinal tract and metabolized.<sup>(11,211,212)</sup> A comparison of the metabolism of BHA in man with that of various animals is presented in Figure 2.

In a study by Astill et al.,<sup>(213)</sup> groups of four or eight male and female Sprague-Dawley rats were administered a single oral dose of 0.4 g BHA/kg. Urinary excretion of the BHA glucuronide and ethereal sulfate during the 5 days after dosing accounted for 61 to 82 and 11 to 16 percent of the dose, respectively; 5 percent of the dose was excreted unchanged. For single oral doses of 0.002, 0.01, 0.025, 0.05, and 0.1 g BHA/kg, overall recoveries of the dose from the urine were 81 to 100 percent. At these doses, glucuronide excretion accounted for 69 to 92 percent of the dose. However, a slight increase in excretion of unchanged BHA occurred at the lowest doses. With single oral dose of 0.4 g/kg of 2-tert-butyl-4-hydroxyanisole (2-BHA), 72 percent of the dose was excreted in 5 days as ethereal sulfate. For the 3-tert-butyl-isomer (3-BHA), 57 to 71 percent of the 0.4 g/kg dose was excreted as glucuronide in 5 days. Administration of

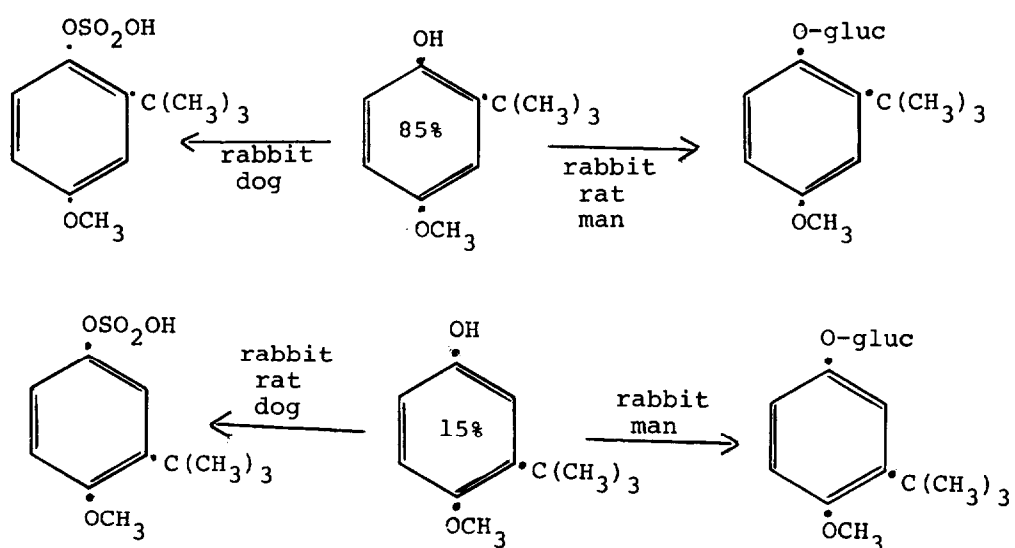


Figure 2. Metabolism of BHA in man and various animals.

**TABLE 4.** Other in Vitro and in Vivo Effects.

<i>BHA Concentration</i>	<i>Test System</i>	<i>Observations/Results</i>	<i>Reference</i>
10 <sup>-3</sup> M for 18 hour exposure	Renal cultures of male rats	Dose-related decrease in glucose metabolism when compared to control values	214
0.0001, 0.001, 0.01 or 0.05 percent	Rabbit intestine heart preparations	Inhibited ileal contractions at 0.001, 0.01, and 0.05 percent, and inhibited atrial contractions at all exposure levels	215
100 ppm (0.55 mM)	Cultured myocardial and endotheloid cells isolated from neonatal rats	Marked leakage of lactic dehydrogenase observed in both myocardial and endotheloid cells. Depressed beating rate of heart cells also observed, with maximum inhibition occurring within 1 hour after BHA exposure. Morphologically, myocardial cells in presence of 100 ppm BHA were similar to control cells; however, when antioxidant concentrations were increased to 1000 ppm (5.5 mM), marked cell lysis occurred	216
0.01 percent in culture media	Cultured myocardial and endotheloid cells	Marked leakage of lactic dehydrogenase	217
2 mg BHA/ml of tissue maintenance media	Perfused rat intestine in situ	Reduced absorption of glucose and methionine by intestine; absorption of butyric acid not affected	218
Total dose of 0.5 g added to diet during 21-day period after mating	Walter Reed Carworth Farm rats-12 litters	Decrease in fetal resorption rates	219
0.1 percent in low cholesterol, atherogenic diet for periods up to 3 years	60 rabbits	Neither hypocholesterolemic nor antiatherosclerotic effects observed	220
5.44 nmol/100g diet for 3 weeks	10 male Sprague-Dawley rats	No hemorrhaging nor deaths observed	221
1000 µg/ml incubation medium	Monkey liver slices	Monkeys were fed 50 mg BHA/kg/day for 1 week, after which they were killed. Lipid synthesis was then determined in liver slices by measuring incorporation of acetate- <sup>14</sup> C into lipids. Both polar and neutral lipid synthesis were over 70% inhibited by addition of 1000 µg/ml of BHA to incubation medium; however, lower levels of BHA inhibited polar lipid formation and stimulated neutral lipid formation. The antioxidant also inhibited synthesis of fatty acids and cholesterol, two neutral lipids directly synthesized from acetate	222
Intraperitoneal injection of 5, 50, or 500 mg/kg dissolved in peanut oil	CF-1 mice	All doses caused large increases in whole-brain concentrations of 5-hydroxyindole acetic acid 24 hours after injection; however, insignificant changes were noted in whole-brain levels of	223

TABLE 4. (Continued.)

<i>BHA Concentration</i>	<i>Test System</i>	<i>Observations/Results</i>	<i>Reference</i>
Single intraperitoneal injection of 62.5, 215 or 500 mg/kg	16–24 Swiss-Webster mice/dose	serotonin and norepinephrine. Results suggested that partial serotonin depletion seen in earlier studies as a result of chronic BHA administration was due to increased serotonin use Dose-dependent increase in lung weight observed 3 days after injection. Although BHA caused some degree of pulmonary edema, the resulting inflammatory response did not appear significant. No changes observed between treated and control animals in lung DNA or nonprotein sulfhydryl content, gross anatomy, or lung activities of superoxide dismutase, glutathione peroxidase, glutathione reductase, or glucose-6-phosphate	209
	Mitochondrial-lysosomal fraction of rat liver	BHA inhibited respiratory function and lipid peroxidation in vitro. Both BHA and BHT dissociated acid phosphatase activity of organelles, but only BHA solubilized glutamic dehydrogenase. Labilization of the lysosomal membrane as well as disorganization or mitochondrial structure also observed.	224–228
1.0 nmol/mg protein	Guinea pig liver homogenate	BHA inhibited cellular respiration	229
	Isolated rat liver mitochondria	BHA inhibited cation efflux during the oscillatory cycle without significant influences on either active transport or oxidative phosphorylation	230
0.8 mM or 1.0 mM	Various biomembrane structures	BHA demonstrated ability to perturb and modify biomembrane structure causing the in vitro lysis of erythrocytes (human, pig, rat), oxidation of hemoglobin, and release of proteins from rat liver mitochondria and lysosomes. It was suggested that the aromatic nucleus, because of its high electron density, is capable of interacting with hydrophobic and electrophilic regions of the membrane	225,231,232
	Human erythrocytes	Authors suggested that BHA may affect human red blood cell function by chelating to hemoglobin	231,232
	Human erythrocytes	BHA (0.8 mM) protected human erythrocytes from hypotonic hemolysis. The antihemolytic activity was a function of the compound's lipid solubility. 50% of red blood cells suspended in isotonic saline hemolyzed when exposed to 1.0 mM BHA. Data suggest that BHA can alter membrane structure and function.	233



	Rats	Oral administration of BHA increased hematocrit value and hemoglobin concentrations in blood; however, these parameters returned to normal 3 days after cessation of BHA treatment	234
10 <sup>-4</sup> M	Rat blood cell suspension	BHA caused considerable hemolysis of blood cell suspension in vitro	234
12.5 µg/ml in culture media	<i>Tetrahymena pyriformis</i> cultures	Inhibition in the synthesis of polar lipids observed, suggesting that BHA may alter membrane fluidity	88
25 to 100 µg	Mouse spleen cells	BHA exerted a significant inhibitory effect on the primary in vitro antibody response of spleen cells to thymus-dependent and independent antigens. The mechanisms of inhibition is unknown but may involve activation of regulatory cell activity	235,236
	Mouse spleen cells	BHA enhanced the in vitro immune responses of spleen cells	237
100 or 300 mg/kg given orally	At least 5 rats/treatment group	Mixed results obtained in tests conducted to determine the anti-inflammatory ability of BHA given orally to rats. Author noted that previous investigators have demonstrated anti-inflammatory activity for BHA	238
50 µmol/L of ethanol	Albumin	BHA inhibited bilirubin oxidation in vitro in both the presence and absence of albumin. BHA did not increase the oxidation rate in the presence of albumin indicating that the antioxidant is not bound competitively to the bilirubin site on the albumin molecule	239
	Mice, mature <i>Drosophila</i> sperm, EL-4 lymphoma cells, AKR leukemia cells, various plants	Exposure of various test systems to BHA indicated that the antioxidant is a potent radiosensitizer	240-244
	Male <i>Drosophila melanogaster</i>	Addition of BHA to culture media increased half-life of irradiated flies	245
0.1 percent BHA/BHT given orally	Syrian hamsters	The antioxidants had no measurable protective effect against radiation-induced pulmonary fibrosis	246
Concentrations of > 30 µg/ml	<i>Tetrahymena pyriformis</i>	Protection against photodynamic toxicity of benzo[a]pyrene observed	247
10 <sup>-4</sup> to 10 <sup>-3</sup> M or 10 <sup>-6</sup> to 10 <sup>-5</sup> M	Chinese hamster Don cells	BHA inhibited mitosis at concentrations of 10 <sup>-4</sup> to 10 <sup>-3</sup> M but not at 10 <sup>-6</sup> to 10 <sup>-5</sup> M	248
20, 25, or 200 ppm	Chicken eggs	The antioxidant was lethal to chicken embryos when injected into the yolk or air cell of the egg	249

**TABLE 4.** (Continued.)

<i>BHA Concentration</i>	<i>Test System</i>	<i>Observations/Results</i>	<i>Reference</i>
0.1 or 1.0% of diet for 8 weeks	Liver homogenate of Wistar rats	Reduced oxygen uptake observed with and without succinate as substrate. Ingestion of BHA at 1% concentration also influenced the phosphorylation efficiency of hepatic mitochondria, with the P/O ratio significantly increased over that of the control group. Results confirmed that BHA influences the oxidoreducing processes at the cell level	250
0.105 or 0.523% of diet	Rats	An increase in the stability of extracted perirenal fat observed	251
Diet supplemented with 10% lard containing 0.01 or 0.5% BHA	Rats	Concentrations of 0.01 and 0.5% BHA lowered and raised the iodine value of fat, respectively	251
25 ppm in dehydrated alcohol	Chicken eggs/chickens	Hypopigmentation of the down of hatched chicks occurred following injection of the antioxidant into either the yolk or air cell prior to incubation. These data suggest a BHA impairment of carotenoid (xanthophyl) metabolism or deposition	249

several successive daily doses of 0.5 g BHA/kg and of 3-BHA decreased the percentage recovery of conjugates and the proportion of ethereal sulfate excreted. Repeated doses of 0.5 g/2-BHA/kg were followed by a considerable increase in glucuronide and a decrease in ethereal sulfate conjugation. The authors suggested that 4-O-conjugation, O-demethylation, hydroxylation of the phenyl ring, and sidechain oxidation ( $-\text{CH}_3 \rightarrow \text{CH}_2 \cdot \text{OH}$ ) 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.

Investigations by Golder et al.<sup>(252)</sup> indicated that the antioxidant is rapidly excreted in urine by the albino rat. Animals given 97  $\mu\text{g}$  of tritiated BHA by intraperitoneal injection excreted 86, 89, and 91 percent of the dose as the conjugated derivative in 24, 48, and 96 hours, respectively. Dacre<sup>(253)</sup> observed that 90 percent of a single dose of 80 to 100 mg BHA given orally to rats was excreted in the urine as glucuronide conjugate (71 percent), ethereal sulfate (14 percent), and unconjugated phenol (5 percent).

Astill et al.<sup>(213)</sup> reported that the metabolism of BHA in the rat resembles that of the rabbit at doses of 0.13 to 0.55 g/kg. They noted that in the rabbit, 2-BHA and 3-BHA are metabolized largely by 4-O-glucuronide formation. In studies by Dacre et al.,<sup>(254)</sup> rabbits given 1.0 g BHA in olive oil by stomach tube (approximately 0.5 g/kg) excreted 46 percent of the dose as glucuronides, 9 percent as ethereal sulfates, and 6 percent as free phenols. The recovery ratios of free phenols, glucuronides and ethereal sulfates after a 0.125 g/kg dose were 19, 84, and 18 percent, respectively. The corresponding ratios after a 0.5 g dose were 4, 60, and 12 percent, respectively.

Gage<sup>(211)</sup> reported that, in dogs, BHA is absorbed in the intestine only to a small extent and excreted after being coupled with sulfuric acid. Other studies confirm these observations. Three dogs were given a 350 mg/kg dose of BHA in lard mixed with the diet. Sixty percent of the antioxidant was excreted unchanged in the feces within 3 days. The remainder of the dose was excreted in the urine as ethereal sulfate (23 percent), t-butyl hydroquinone (5.5 percent), free BHA (3.6 percent), and as an unidentified phenol.<sup>(255)</sup> Fasted dogs given dietary BHA doses of 50 and 250 mg/kg in the diet had ethereal sulfate and glucuronides in the urine.<sup>(256)</sup>

In order to characterize the different metabolic pathways of BHA in humans, a single 100 mg oral dose of the antioxidant in gelatin capsule was given to an unreported number of patients. Less than 1 percent of the administered dose was recovered in the urine as intact compound, with the bulk of the dose eliminated as glucuronides (about 44 percent), sulfates (about 26 percent), and O-demethylated metabolites (about 42 percent).<sup>(174)</sup> In a second study, a single dose of 40 mg  $^{14}\text{C}$ -labeled BHA was administered orally to two men. This dose approximated the figure of 0.5 mg/kg recommended by the Joint FAO/WHO Expert Committee on Food Additives as the maximum acceptable daily intake for man. In 2 days, 60 to 73 percent of the radioactivity was excreted in the urine; within 11 days, 80 to 87 percent 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.<sup>(257)</sup> In a third study, six adult men were given a single 0.4 to 0.7 mg/kg oral dose of BHA either by capsule (50 mg) or by oil-milk emulsion (31 mg). Twenty seven to 77 percent of the dose was excreted in the urine as glucuronide within 23 to 38 hours. Less than 1 percent of the administered doses appeared in the urine as

ethereal sulfates or as free BHA, and no dealkylated or hydroxylation products were detected. The time required for excretion of the administered dose varied from 23 to 50 hours.<sup>(255)</sup>

The Select Committee on GRAS Substances<sup>(11)</sup> suggests that tissue storage of BHA may occur because of the antioxidant's lipid solubility. However, they note that the amount stored may be quite limited because of rapid metabolism and excretion. This view is supported by studies in which pigs fed 0.1 percent BHA in the diet for 4 months and pullets fed 0.1 percent BHA in the diet for 8 weeks, showed no accumulation of the antioxidant in muscle, liver, kidney, or the reserve fat.<sup>(258)</sup> In groups of dogs maintained for 1 year on diets containing BHA at 0.3, 3.0, 30, and 100 mg/kg as a 50 percent solution in propylene glycol, there was no storage of BHA in the body fat, brain, liver, or kidney.<sup>(259)</sup> In addition, Wilder and Kraybill<sup>(260)</sup> found only trace amounts of BHA in depot and carcass fat or rats given 2 to 3 percent of the antioxidant in the diet for 6 months.

Pharmacokinetic studies with rabbits revealed that, despite extensive distribution of BHA following a single intravenous 10 mg/kg dose, "fast clearance" of the antioxidant is achieved because of the "highly reversible nature of its storage in body tissues." A "disposition half-life" of approximately 1 hour was estimated for BHA in these studies. According to the investigators, data obtained with the rabbit model suggest that the human body behaves as a "homogeneous compartment" for BHA in terms of "equilibrium rates among various body tissues."<sup>(261)</sup>

The binding of BHA to human albumin was studied in vitro. The percent binding of BHA as a function of total concentration in the "protein compartment" ranged from a minimum of 68 percent to a maximum exceeding 90 percent. The magnitude of the binding constant ( $2.4 \times 10^4$  to  $2.9 \times 10^4$ ) suggested that any change in protein binding will have a significant effect on the distribution of the antioxidant throughout the body. Such changes may be brought about by variations in the amount of BHA ingested. It was noted that ingestion of a 2 mg/kg dose yields a "maximum concentration" of approximately 26  $\mu\text{g/ml}$ , assuming all the BHA initially remains in the "blood compartment." Thus, during normal ingestion of the antioxidant, a "high degree of protein binding can be assumed."<sup>(262)</sup>

## Animal Toxicology

### Acute Oral Studies

The acute oral toxicity of BHA has been determined in both rats and mice (Table 5).

Cosmetic products containing BHA have also been evaluated for their acute oral toxicity. A suntan preparation containing 0.1 percent BHA and an eye shadow containing 0.2 percent of the antioxidant were given by oral intubation to female albino rats in two separate studies. The products were administered to 10 rats (5 animals/product) in a single 5.0 g/kg dose. Animals appeared normal throughout the 7 days of observation following administration in both tests, and no gross lesions were found at necropsy. The acute oral  $\text{LD}_{50}$  for both products was  $> 5.0 \text{ g/kg}$ .<sup>(268,269)</sup> An eye makeup preparation containing 0.1 percent of the antioxidant was evaluated for acute oral toxicity in a third study by means of the procedures outlined in 16 CFR: 1500.3 (b) (6) (i) (A). The product  $\text{LD}_{50}$  in rats was also  $> 5 \text{ g/kg}$ .<sup>(270)</sup>

TABLE 5. Acute Oral Toxicity.

<i>Material Tested</i>	<i>Animal</i>	<i>LD<sub>50</sub></i> <i>(g/kg)</i>	<i>Reference</i>
BHA in olive oil	Male and female rats	2.0	263
BHA	Rats	2.2	253,264
BHA in isopropyl alcohol	Male rats	2.8	265
BHA	Rats	2.9	191,253
BHA	Male rats	2.95	190
BHA in corn oil	Nonfasted rats	4.1	253,266,267
BHA in water	Fasted rats	> 5.0	253,266,267
BHA in olive oil	Male mice	1.10	263
BHA	Mice	1.25	191,253
BHA in olive oil	Female mice	1.32	263
BHA	Mice	2	253,264,267

### Eye Irritation

The eye-irritating ability of a face powder containing 0.2 percent BHA was studied in six albino New Zealand rabbits. The test material was instilled into one eye of each animal in a single 0.1 ml dose; the untreated eye of each rabbit served as control. It was not reported whether or not the treated eyes received a water rinse following instillation of the test material. Average eye irritation scores 24 and 48 hours following exposure to the face powder were 2 and 0, respectively (the maximum possible score per observation was 110). The investigators concluded that the product was a "minimal" eye irritant.<sup>(271)</sup>

A second group of six albino New Zealand rabbits was used to test the eye-irritating ability of an eye shadow containing 0.2 percent BHA. The test material (0.1 ml) was instilled three times a day for an unspecified number of days into the conjunctival sac of one eye of each animal by means of a 4-second spray held 6 inches from the face. It was not reported whether or not the treated eyes were given a water rinse. Average eye irritation scores on Days 1, 2, 3, 4, and 7 were 2, 1, 2, 1, and 0, respectively (maximum score per reading, 110). The product was considered a "mild" eye irritant by the investigators.<sup>(272)</sup>

A third eye irritation test was conducted with an eye makeup preparation containing 0.1 percent BHA according to the methods outlined in 16 CFR 1500.42. One-tenth ml of the test material was placed into one eye of each of six albino rabbits in a single application; the untreated eyes served as controls. Treated eyes did not receive a water rinse. Eyes were graded for ocular reaction 24, 48, and 72 hours following instillation of the test preparation. All treated eyes were negative for conjunctival redness, conjunctival chemosis, keratitis, and iritis.<sup>(270)</sup>

### Skin Irritation

A suntan preparation containing 0.1 percent BHA and an eye shadow and a face powder containing 0.2 percent of the antioxidant were evaluated for their skin-irritating ability in three separate tests. In each study, the test formulation (0.1 ml) was applied under an occlusive filter disc to the clipped skin of the back of each of 9 albino rabbits. The discs were removed after 24 hours of skin con-

tact, and the test sites were graded for irritation on a scale of 0 (no effect) to 4 (severe irritation). The grading of skin reactions was repeated 48 to 72 hours following 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.<sup>(273-275)</sup>

An eye makeup preparation containing 0.1 percent BHA was evaluated in a fourth skin irritation study by means of the procedures specified in 16 CFR 1500.41. The test material (0.5 g) was applied under an occlusive patch to the intact and abraded skin of each of 6 albino rabbits. After a 24-hour exposure period, the patch was removed, and the test sites were graded for irritation. Skin reactions were evaluated again 48 hours following the first reading. The primary irritation index was 2.75, indicating that the product was a moderate skin irritant.<sup>(270)</sup>

### **Percutaneous Toxicity/Dermal Irritation**

Two guinea pig immersion tests were conducted to evaluate percutaneous toxicity and dermal-irritating ability of two different bubble bath formulations each containing 0.1 percent BHA. Six albino guinea pigs in each study were clipped free of abdominal hair and immersed up to their axillae in a 0.5 percent aqueous solution of the product. The 12 animals (6 animals/product) were exposed to actual BHA concentrations of 0.0005 percent ( $0.005 \times 0.1$  percent) 4 hours a day for 3 consecutive days. Forty-eight hours following the last exposure, the skin reactions on the abdomen were graded on a scale of 10 (normal skin) to 1 (moribund due to skin injuries). The average irritation indices for the two formulations were 7.9 and 5.0, 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.<sup>(276,277)</sup>

### **Acute Dermal Toxicity**

An unspecified number of rabbits were used to evaluate the acute dermal toxicity of an eye makeup preparation containing 0.1 percent BHA. The test procedure employed was that as outlined in 16 CFR 1500.40. The dermal LD<sub>50</sub> of the makeup formulation was determined to be  $> 2$  g/kg.<sup>(270)</sup>

### **Acute, Subchronic, and Chronic Oral Administration**

A number of short and long-term oral administration studies were conducted on a variety of animals, including rats, rabbits, chickens, dogs, guinea pigs and monkeys (Table 6). The effect of BHA on such factors as growth, survival, behavior, organ weights, blood counts, blood chemistries, enzymatic activity, electrolyte balance, fat metabolism, hormonal activity, sex cycle, reproduction, and function of liver, kidney, adrenal, and reproductive glands varied according to test conditions and to dosage and duration of antioxidant administration. One frequent finding was enlargement of the liver and/or increased liver weight; however, these changes were not generally accompanied by persistent hepatotoxicity.

**TABLE 6.** Acute, Subchronic, and Chronic Oral Administration.

<i>BHA Dose</i>	<i>Animal</i>	<i>Results</i>	<i>Reference</i>
Administered by stomach tube at 500 mg/kg/day in peanut oil for 84 days	4 or 5 female Carworth Farm SPF rats	Activities of hepatic hexobarbital oxidase, nitroanisole demethylase, codeine demethylase, and aminopyrine demethylase not affected; however, significant increases in liver weight and liver protein observed. Investigators thought it appropriate to disregard hyperfunctional liver enlargement in assessing the acceptability of BHA as a food ingredient	196
0, 50, 100, 200, or 500 mg/kg/day for 7 days by stomach tube	4 to 12 male and female SPF Carworth rats/dose group	No changes in liver fat noted; however, significant increases in liver weights observed in females at 50, 200, and 500 mg/kg/day and in males at 100, 200 and 500 mg/kg/day	284
In diet at 500 mg/kg/day for 82 days or 600 mg/kg/day for 68 days	Rats	Lag in weight gain and reduced blood catalase and peroxidase activities observed. Increases in liver weights and body fat content observed; however, no pathologic differences noted between treated and control animals at autopsy	191
4 mg/kg/day in diet for 30 to 35 days	Rats	No changes observed with respect to fat metabolism, weight gain, liver glycogen, cholesterol, phospholipids, or concentration and iodine number of liver fat	285
400 mg/kg/day in diet for 4 to 8 weeks	8 rats	No change in various blood characteristics except for a decrease in phagocytic activity of leukocyte	286
0, 0.1, 0.2, 0.3, 0.4, or 0.5% of diet for 6 weeks	6 male and 6 female Norway hooded rats/dose group	Increased levels of total serum cholesterol noted in animals fed 0.1%. Associated with increasing dietary concentrations of the antioxidant were increases in both the absolute lipid content and weight of the liver. Although increases in male adrenal weights also associated with increasing dietary concentrations of BHA, no histologic changes attributable to treatment could be detected. No changes 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	287
500 mg/kg/day for 6 days by gavage	4 male Sprague-Dawley rats	No differences in the fluid balances or in the osmolality of urine between BHA-treated and control animals; however, urinary sodium and potassium concentrations reduced in treated animals. Total daily sodium excretion of treated animals less than expected from food intake on Days 2-6, possibly owing to interference with renal prostaglandin synthesis	288
500 mg/kg/day in diet for 1, 2, 4, or 6 days	Male rats	<i>p</i> -Aminohippurate (PAH) accumulation was reduced in the kidney after one dose of BHA. Increases in liver weight following second dose accompanied by increases in the PAH serum to slice ratio, but these increases approached normal levels after six doses	289

TABLE 6. (Continued.)

<i>BHA Dose</i>	<i>Animal</i>	<i>Results</i>	<i>Reference</i>
1 g in olive oil given by stomach tube for 4 to 7 days	Rabbits	Dose lethal to animals	254
1 g in olive oil given by stomach tube for 1 to 7 days	Rabbits	Authors established that lethal effect of such large doses of BHA due to excessive loss of potassium in urine, and marked decreases in potassium of muscle and other tissues. Depletion of lipid in the zona glomerulosa of the adrenal was also observed. The underlying cause for the excessive loss of electrolytes, and for the "gross disturbance" of salt (potassium and sodium) and water balance was not established; however, the author's suggested the primary effect of BHA may have been renal	290
0.37% (250 mg/kg body weight/day) in low-fat or high-fat/high-cholesterol ration for 12 weeks	Chickens	Low-fat ration: no observed effects on growth, blood lipid levels, or liver lipid levels. High-fat/high-cholesterol ration: increased levels of serum lipids and decreased liver lipids. Mechanism by which antioxidants prevent fatty livers suggested to involve prevention of lipid peroxidation either directly, or by sparing vitamin E	291
500 mg/kg/day for 4 weeks by gastric intubation	Adolescent rhesus monkeys	Weekly blood counts, electrolyte determinations, and liver function tests revealed no significant changes, nor were there significant changes in microsomal levels of RNA, phospholipids or cytochrome P <sub>450</sub> . After 4 weeks, activities of glucose-6-phosphatase and nitroanisoie demethylase were reduced and elevated, respectively. 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	292
50 or 500 mg/kg/day for 28 days by gastric intubation	9 infant and 17 juvenile rhesus monkeys of both sexes given 500 mg/kg, and 17 juvenile rhesus monkeys of both sexes given 50 mg/kg	In juvenile monkeys given 500 mg/kg/day, a transient decrease in serum cholesterol levels and a pronounced increase in relative liver weights was observed. Monkeys given 50 mg/kg/day also showed enlarged livers, but size of increase was of questionable significance. Histologic evaluations of "all organs other than liver" failed to reveal ingestion-related pathologic changes in infants or juveniles. Histologic evaluation of the liver of juveniles revealed cytomegaly and enlargement of cell nucleoli. Both infants and juveniles of high-dose group showed pronounced proliferation of hepatic endoplasmic reticulum and an increase in the number of cytoplasmic lipid droplets; fragmentation of the nucleolus and coarse nucleolar fibrils also observed. Similar nucleolar changes not seen in	210



		low-dose group. No significant variations observed in cytochrome P <sub>450</sub> levels, or hepatic protein, DNA, or RNA. Nitroanisole demethylase and glucose-6-phosphatase showed increased and decreased activities in juveniles of the high-dose group, respectively, whereas, no appreciable changes in activities of these enzymes noted in liver microsomes of infants	
0, 50 or 500 mg/kg/day in diet for 28 days	2, 3, and 4 monkeys given 0, 50, and 500 mg/kg, respectively	Total cholesterol levels in plasma and liver significantly lowered at the 500 mg/kg/day dose; treatment at 50 mg/kg/day significantly lowered liver cholesterol. Lipid-phosphorus levels in the plasma significantly increased by 500 mg/kg/day, and all animals receiving this latter dose and the lower dose had lowered cholesterol and lipid-phosphorus ratios in the plasma and liver. Investigators 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	293
BHA (in commercial antioxidant preparation) given in diet at a level of 1.35 or 67.5 mg/kg of diet for 32 or 52 weeks	Wistar albino rats of both sexes	No deleterious effects noted in either trial with respect to survival, growth, organ weights (liver, heart, kidney, spleen), or hemoglobin level. Histopathologic studies of the brain, lungs, heart, liver, spleen, stomach, intestine, kidney, adrenal, bladder, testis, trachea, thymus, and pancreas revealed no changes that could be attributed to the antioxidant preparation	281,294
0.0, 0.0004, 0.001, 0.1, or 0.5% of diet for 8 months (Norway rats); or 0.01 or 0.1% of diet for 2 years (albino rats)	Total of 80 hooded Norway rats and a total of 26 albino rats	A reduction in mature weight and an increase in relative liver weight noted at the 0.5% level. BHA had no effect at any level on hooded or albino rats with respect to mortality, reproductive cycle, hair condition, histology of spleen, testes, kidney, liver, and skin, or relative weights of spleen, heart, and kidney. Toxicity of BHA not affected by dietary fat load	282
0.1% of diet for periods up to 16 weeks	24 male and 24 female weanling Carworth SPF rats of both sexes	An increase in urinary ascorbic acid excretion was observed in animals at 4 weeks. Growth closely paralleled controls except toward the end of the 16-week period, where there was a retardation of growth in males together with a decrease in food consumption. Increases in relative liver weight occurred predominately in females. In a few instances, increases in adrenal weights were also seen in females. No significant changes in hepatic glucose-6-phosphatase occurred in either sex after 16 weeks; however, a decrease in this enzyme's activity was noted in females after 4 weeks. There was no histopathologic evidence of damage to the liver in any animal	194
4 mg/kg/day in diet for 6 months	Albino rats and guinea pigs	Rats showed transient eosinopenia beginning in the 5th month. Guinea pigs showed a temporary drop in urinary 17-oxycorticosteroids after 4 months. Neither rats nor guinea pigs showed impaired function of the reproductive glands. There was also no observed adverse effects to either	120

TABLE 6. (Continued.)

<i>BHA Dose</i>	<i>Animal</i>	<i>Results</i>	<i>Reference</i>
Mixture of propyl gallate (20 mg/kg/day) and BHA (10 mg/kg/day) for 9 months in diet	Female rats	animal with respect to sex cycle phases, activity of gonadotropic pituitary hormone glands, or histology of endocrine glands Female rats failed to produce progeny when mated with similarly fed males	190
0.0004, 0.001, 0.1, or 0.5% in diet for 8 months	Total of 80 Norway hooded rats	No deleterious effects on reproduction in terms of 21-day litter weights or numbers of pups born and weaned	282
2.0% of diet for 6 months or 0.12% of diet for 21 months	6 rats (6 months) and 68 weanling rats (21 months), respectively	Weight gains reduced in rats fed BHA for 6 months; however, histopathologic examination revealed no adverse effects attributable to the antioxidant. Rats fed BHA for 21 months showed no significant differences from controls, with respect to weight gain, growth, reproduction, or histopathology	260,266
0; 0.05, 0.15, 0.45, or 1.35% of diet for 110 days	20 rats/dose	Increased serum protein noted in males at all dose levels and in females given 1.35%. White blood cell counts elevated in males given diets containing 0.05 and 0.15% BHA, whereas hemoglobin levels reduced in females receiving 1.35% of the antioxidant. Red blood cell count, differential leukocyte count, and hematocrit level of both sexes were comparable to those of controls at all dose levels. Males at each dietary concentration exhibited increased liver weight but normal weights for brain, pituitary, thyroid, thymus, heart, testis, prostate, spleen, and adrenal; 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 at each dietary level of BHA comparable to those of 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%).	263
0, 5, 50, or 250 mg/kg/day in diet for 15 months	4 Weanling cocker spaniel dogs/dose	No appreciable differences between control and BHA-fed dogs observed throughout the study with respect to appearance, behavior, hemoglobin levels, red and white blood cell counts, or differential white cell counts. Urine from dogs fed BHA contained higher levels of glucuronates as compared to controls, as well as a higher ratio of total to inorganic sulfates. Normal weight gain and food consumption observed in dogs fed	256

<p>In diet as a 50% soln. in propylene glycol at a level of 0, 0.3, 3.0, 30, or 100 mg/kg/day for 1 year; or orally at 30 mg/kg/day for 1 year in soln. containing 20% BHA, 6% propyl gallate, 4% citric acid, and 70% propylene glycol</p>	<p>3 beagle dogs/dose</p>	<p>5 or 50 mg/kg/day; however, dogs given 250 mg/kg/day gained less weight and consumed less food per day when compared to controls. Microscopic examination of heart, liver, lung pancreas, spleen, kidney, intestine, lymph node, stomach, thyroid, parathyroid, adrenal, gonad, and bone marrow at the end of the 15 months revealed "no changes beyond normal variation" in any of the animals with the exception of the high-dose group (250 mg/kg/day). Three of four dogs in this group showed liver parenchymal degeneration, as well as diffuse granulocytic infiltration accompanied by marked narrowing of hepatic sinusoids. Accumulation of bile pigment in periportal areas and increased hemosiderin in Kupffer cells were also observed. It was noted that these three dogs each consumed more than 1.5 g BHA/day during the 15-month period. The fourth dog in the high-dose group consistently ate only half of the daily ration (786 mg BHA/day or 183 mg BHA/kg). The author suggested that reduced daily intake of BHA may have accounted for the absence of liver degeneration in this dog</p>	<p>259</p>
<p>Both 50 mg/kg BHA and 50 mg/kg BHT in diet for 2 years</p>	<p>6 adult rhesus female monkeys</p>	<p>The dogs remained in "good condition" throughout the study, and there were no deaths. Body weights increased slightly or were maintained in every dog but 1. Urine analyses at the start and end of the study showed normal values for protein and sugar. Blood samples taken on nine occasions during the study gave normal values for hemoglobin level. Organ weights (liver, kidney, lungs, brain, heart, spleen) for each test group were within normal ranges. There was no storage of BHA in fat, brain, liver, or kidney, and there was no increase in urinary reducing substances. 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 any tissue change attributable to BHA</p> <p>During the first year, there were no abnormalities in exposed animals as compared to controls with respect to hemograms, blood chemistry, menstrual cycles, food consumption, or body weight. Following the initial year of exposure, the monkeys were bred to rhesus males that had received unmodified diets. Gestation was free of complications and normal infants delivered to treated animals. Hematologic evaluations of exposed animals throughout gestation and for 60 days thereafter were similar to those of control infants. The infants and adults were observed for 2 years following the antioxidant exposure. During this time, the adult females continued to have normal infants and the infants born during the exposure period remained healthy</p>	<p>295</p>

## Carcinogenesis

No evidence of carcinogenicity was observed during an observation period of 273 to 575 days in 100 C3H/Anf mice (50 of each sex), each given a single subcutaneous injection of 10 mg BHA in trioctanoin. When two similar groups of mice were given weekly skin applications of either 0.1 or 10 mg BHA in acetone, there was no gross or microscopic evidence of skin tumor formation after 323 to 519 days.<sup>(278)</sup>

Berry et al.<sup>(279)</sup> topically applied 1 mg BHA in acetone solution to the shaved backs of 30 female CD-1 Charles River mice twice weekly for 30 weeks. Weekly histologic examination by the end of the experimental period revealed no papillomas or carcinomas. BHA was not a tumor promoter in a second group of 30 mice treated similarly following a 1-week initiation period with topically applied 7,12-dimethylbenz[a]anthracene (200 nmol).

A/He mice were given BHA in 0.1 ml tricaprylin by intraperitoneal injection at doses of either 1.2 (30 mice) or 6.0 (30 mice) g/kg. Doses were administered three times a week for 8 weeks. Twenty-four weeks after the first injection, the animals were killed and the lungs examined grossly and microscopically. Liver, kidney, thymus, intestine, salivary glands, and endocrine gland were also examined at necropsy. No significant differences were observed between treated animals and untreated or vehicle controls in terms of tissue abnormalities or number of pulmonary tumors.<sup>(280)</sup>

Rats were given diets containing BHA at levels of either 0, 1.35, or 67.5 mg/kg of diet for 1 year. Two fibroadenomas were noted in 13 female rats on one of four diets at the higher level.<sup>(281)</sup> Rats given diets containing 0, 0.003, 0.03, 0.06, or 0.12 percent BHA for 21 to 22 months had no tumors or other pathologic changes.<sup>(260)</sup> No tumors were reported in rats fed diets containing 0, 0.01, or 0.1 percent of the antioxidant for 2 years (Table 6).<sup>(282)</sup>

Dietary administration of BHA to dogs at doses of 0, 5.0, 50, or 250 mg/kg/day for 15 months,<sup>(256)</sup> or concentrations of 0, 0.001, 0.01, 0.1, or 0.3 percent for 1 year<sup>(259)</sup> caused no carcinogenic effects (Table 6).

Results from one recent study suggest that BHA is carcinogenic to the forestomach of rats.<sup>(283)</sup> The antioxidant was incorporated into the basal diet of F344 rats at 0.5 and 2.0 percent for 2 years. Analyses of food samples on five occasions showed that the actual concentrations of BHA in food containing 0.5 and 2.0 percent were 0.24 and 1.07 percent, respectively. In both males and females of the high-dose group, an increased incidence of papillomas and squamous cell carcinomas of the forestomach was observed. Animals from each dose group also showed an increased incidence of forestomach hyperplasia. These neoplastic changes were dose-dependent. Benign and malignant tumors were found in other organs of BHA-treated rats as well, but their incidence was not significantly different from that occurring in control animals. Survival, behavior, red and white blood cell counts, and urinalysis of BHA-exposed animals were similar to controls. Platelet counts of females from both treatment groups were significantly higher than controls. Blood chemical analyses revealed that total protein was increased in BHA-treated females. A dose-related decrease in the albumin:globulin ratio was also observed in BHA-treated females. In the high-dose group, mean body weights of both sexes after 16 weeks, as well as food intake of females, were lower than controls. Dose-related decreases in the absolute brain weight of males given BHA and dose-related increases in the relative

weights of the salivary glands and heart of females given BHA were observed. BHA-treated rats also showed a dose-related chronic interstitial nephritis and a lowered incidence of bile duct proliferation. This study is currently under scientific review by the US Food and Drug Administration.<sup>(110)</sup>

### Mutagenesis

BHA at 0.0075 percent had no mutagenic activity against *S. typhimurium* (TA-1535; TA-1537; TA-1538) or *Saccharomyces cerevisiae* (D4) when tested in a series of in vitro assays, with and without the addition of mammalian metabolic activation preparations.<sup>(296)</sup> Similar results were obtained with an analogous assay system, with and without the addition of microsomal mixed function oxidases from rat liver, BHA at 10, 100, and 1000  $\mu\text{g}$  per plate, and *S. typhimurium* strains TA-98, TA-100, TA-1535, TA-1537, and TA-1538.<sup>(297)</sup>

The antioxidant did not induce mutations in a host-mediated assay when tested against *S. typhimurium* (TA-1530 and G-46) at in vivo doses of 15, 150, and 1500 mg/kg. However, significant increases in recombinant frequencies occurred at each of these doses in a host-mediated assay involving *S. cerevisiae* (D3).<sup>(265)</sup>

BHA at doses of 15, 150, and 1500 mg/kg in isopropyl alcohol was non-mutagenic in a dominant lethal study with rats. The test material was administered orally by intubation either in a single dose or daily for 5 days.<sup>(265)</sup>

In cytogenetic studies, no significant aberrations of bone marrow metaphase chromosomes were noted in rats administered 15, 150, or 1500 mg/kg BHA in either a single oral dose or five oral doses 24 hours apart. The compound in isopropyl alcohol at concentrations of 2, 20, or 200  $\mu\text{g}/\text{ml}$  produced no significant aberration in the anaphase chromosomes of human embryonic lung cultures in vitro.<sup>(265)</sup> Chromosomal aberration tests conducted on Chinese hamster fibroblasts in vitro were negative for  $10^{-4}\text{M}$  BHA in ethanol.<sup>(298)</sup>

Natake et al.<sup>(299)</sup> confirmed by rec-assay that "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, but this compound was nonmutagenic in the Ames test with *S. typhimurium* (TA-1535; TA-98). On the other hand, Ishizaki et al.<sup>(300)</sup> found that 2-tert-butyl-hydroquinone, a degradation product resulting from BHA exposure to UV radiation, was mutagenic in assays with wild and recombinationless strains of *Bacillus subtilis* and wild and rad mutant strains of yeast.

Miyagi and Goodheart<sup>(301)</sup> reported that ingestion of 0.01 to 0.15 percent BHA in 5 percent ethanol or 1 percent sucrose solution by *Drosophila melanogaster* yielded no higher frequency of sex-linked recessive lethals in mature spermatozoa than in control flies. The antioxidant was also found nonmutagenic in a second sex-linked recessive lethal test when fed over a 72-hour period to *D. melanogaster* at concentrations of 5 percent in a carrier compound of butter and 2 percent glucose.<sup>(302)</sup>

Thomas et al.<sup>(303)</sup> found 0.2 mM BHA to be a "potent" enhancer of nitrous acid mutagenesis of duplex DNA in *Haemophilus influenzae*.

### Teratogenesis

Hansen and Meyer<sup>(304)</sup> administered BHA by gavage to pregnant SPF New Zealand rabbits at doses of 0, 50, 200, and 400 mg/kg/day from Day 7 to 18 of the

gestation period; 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, and on the weight of fetuses.

Hansen et al.<sup>(304)</sup> also studied the embryotoxicity of BHA on Danish Landrace SPF pigs. Animals were divided into four groups and then fed BHA from time of mating (artificial insemination) to Day 110 of gestation at doses of 0, 50, 200, and 400 mg/kg/day. The number of pregnant gilts in the four groups totaled 9, 11, 13, and 10, respectively. Fetuses were removed by caesarean section on gestation day 110 and subsequently examined for visceral and skeletal defects. BHA neither affected reproduction as measured by pregnancy rate, number of implantations, and number of corpora lutea, nor showed any significant teratogenic effect. Major visceral and skeletal defects observed in the experiment were "within normal range" for Danish Landrace pigs. Necropsy of dams revealed only "sporadic and common pathologic lesions" but no changes in reproductive organs. 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/day. Hematologic parameters (hemoglobin, packed cell volume, total erythrocyte count, reticulocyte count, and differential leukocyte count) were "within the 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. Iodine content from fixed thyroid tissue of both the control and high-dose group was 0.9 mg/100 g tissue, indicating that the alterations observed in the thyroid were not due to a shortage of iodine in the diet or to any interference with iodine uptake. Although no influence of a possible reduced activity was reflected in any of the parameters studied, the authors suggested that the histologic changes in the thyroid gland indicated a reduced thyroidal activity.

Oral intubation of up to 225 mg/kg/day BHA to CD-1 mice from Day 6 to Day 15 of pregnancy had no discernible effect on nidation or on maternal or fetal survival. 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. Negative teratogenic results were also reported for Wistar-derived albino rats receiving up to 200 mg/kg/day from Days 6 through 15 of pregnancy and for hamsters receiving 120 mg/kg/day from Days 6 through 10 of pregnancy.<sup>(305)</sup>

Intragastric administration of BHA to rabbits at doses up to 200 mg/kg on Days 6 through 18 of pregnancy had an adverse effect on the survival of both dams and fetuses. This adverse effect did not appear to be a graded response related to dose. 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 the number occurring spontaneously in the sham-treated controls. The investigators concluded that BHA, while exhibiting systemic toxicity to the rabbit at the range of dosage employed, was not a teratogen.<sup>(306)</sup>

Clegg<sup>(307)</sup> administered BHA in arachis oil by oral intubation to ICI SPF female mice and females of four rat strains (Tuck, Carworth SPF, Porton albino, and Bengel hooded). The following dosage regimens were used: (1) ICI SPF

mice—daily administration of 500 mg/kg for 7 weeks before pairing and continuing until Day 18 of pregnancy, (2) Tuck albino rats—daily administration of 750 mg/kg on Days 1 to 20 of pregnancy, (3) Tuck albino and Benger hooded rats—daily administration of 750 mg/kg for 70 days before pairing and continuing through pregnancy, (4) Tuck and Carworth SPF albino rats—single administration of 1000 mg/kg on Day 9, 11, or 13 of pregnancy, and (5) Porton albino rats—daily administration of 500 mg/kg for 7 weeks before pairing and continuing during pregnancy; treatment commenced when rats were 3 or 10 weeks old. All doses retarded growth of weanling albino female rats and produced weight loss in adults. However, no significant embryotoxic or teratogenic effects as judged by a number of criteria were seen in any strain of either species (albino or hooded). The abnormalities that were encountered were considered spontaneous, since they occurred in untreated as well as treated groups.

## Clinical Assessment of Safety

### Case Reports of Hypersensitivity

The North American Contact Dermatitis Group (NACDG) reported the incidence of skin sensitization among 548 subjects exposed to 2 percent BHA to be 2 percent (11 subjects).<sup>(308)</sup>

A 52-year-old woman developed contact dermatitis of the face following use of a cosmetic formulation. The offending allergen was subsequently identified in a patch battery as BHA. The patch test concentration of this agent was 0.1 percent in soft paraffin, whereas the commercial product she had been using contained 0.005 percent BHA. Since avoiding the product, the patient has had no further problems.<sup>(309)</sup>

A 32-year-old woman acquired dermatitis following use of a hand cream formulation. BHA proved to be the causal agent of her allergic dermatitis.<sup>(82)</sup>

A 48-year-old male cook developed allergic contact dermatitis of the hands and circumoral area after contact with mayonnaise containing BHA. Patch tests with the mayonnaise were positive in the patient and negative in three controls. The positive patch test was obtained with 2 percent BHA, the suspected allergen. Avoidance of mayonnaise prevented the recurrence of hand and circumoral dermatitis.<sup>(310)</sup>

Degreff and Verhoeve<sup>(311)</sup> observed one case of contact sensitivity resulting from the use of an antimycotic cream containing 0.052 mg BHA. Subsequent patch test results were positive for both 5 percent BHA in petrolatum and the cream's active ingredient (miconazole nitrate).

One hundred twelve patients referred to a clinic for eczematous dermatitis of different types, caused by various creams, were patch tested with 2 percent BHA in petrolatum. Three of these patients were positive for contact dermatitis. Biopsy results and control patch tests confirmed that the reactions were allergic and not irritant.<sup>(312)</sup> This study demonstrates a lymphocyte-mediated allergy in which patients have been sensitized after repeated local application of the chemical.<sup>(313)</sup>

Eighty-three "consecutive" patients with eczematous dermatitis were patch tested with 5 percent BHA in alcohol; all were negative for contact dermatitis.<sup>(312)</sup>

Fisherman and Cohen<sup>(314)</sup> identified seven patients with suspected sensitivity to BHA and BHT. These patients showed exacerbated signs of allergy when given oral doses of 125 to 250 mg BHA or BHT following 12 hours 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 percent or more also occurred in BHA- and BHT-sensitive patients after oral challenge but not in control subjects. No rationale was cited for the effect of BHA on bleeding. Fisherman et al.<sup>(315)</sup> reported other studies in which 37 subjects were identified as BHA- and BHT-intolerant.

Fisher<sup>(82)</sup> described two patients who had dyshidrotic eczema that cleared when they were placed on a BHA- and BHT-free diet. These patients were subsequently challenged orally with BHT and BHA and within 12 hours had vesicles on their hands and lips.

Daily oral administration of 5 or 10 mg BHA for 4 days caused a flare-up of skin dermatitis in BHA- and/or BHT-sensitive individuals.<sup>(312)</sup>

A 32-year-old patient reacted with generalized urticaria in a double-blind study following ingestion of BHA and BHT. Cryoglobulins, histamine levels, total hemolytic complement, and complement split products were elevated in the serum. The patient had a persistent cryoglobulinemia that did not change with "challenge" and normal baseline histamine concentrations that elevated with challenge to BHA and BHT. Complement split products did not elevate during the challenges.<sup>(316)</sup>

### **Skin Irritation, Sensitization, and Photosensitization**

Numerous clinical studies have been conducted to determine the abilities of BHA and cosmetic products containing BHA to cause skin irritation, sensitization, and photosensitization. These studies are discussed below, and results are summarized in Table 7. Conclusions stated in Table 7 are as reported by the investigator.

Five separate studies were conducted to evaluate the skin-irritating ability of two cosmetic products containing 0.1 percent BHA and three cosmetic products containing 0.2 percent of the antioxidant. In each of the five studies, the test material was applied under an occlusive patch in a single 0.1 ml dose of the volar surface of the forearm and/or inner aspect of the upperarm of 19 to 20 subjects. Ages of those tested ranged from 18 to 65. After 24 hours, the patches were removed and the test sites graded for skin irritation on a scale of 0 (no irritation) to 4 (severe irritation). For the three products each containing 0.2 percent BHA, the primary irritation indices\* were 0.03 (20 subjects), 0.05 (20 subjects), and 0.15 (20 subjects), respectively, indicating in each case minimal skin irritation.<sup>(317-319)</sup> Primary irritation indices of 0 (20 subjects) and 0.95 (19 subjects) were reported for the two formulations containing 0.1 percent of the antioxidant, indicating no skin irritation and minimal/mild skin irritation, respectively.<sup>(320,321)</sup>

Three different shave cream formulations each containing 0.01 percent BHA were studied for their ability to induce primary skin irritation and sensitization. The three products were evaluated in separate tests on groups of 50, 54, and 57 adult subjects, respectively. In each study, an occlusive 12-hour patch containing the test material was applied to the medial surface of the upper arm. Each panelist received patches 4 successive days per week for 2 weeks for a total of

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\* The primary irritation index (PII) is a value depicting the average skin response of the test panel as a whole. It is calculated by adding the irritation scores and dividing by the total number of test subjects.



**TABLE 7.** Clinical Studies.

<i>Type of Study</i>	<i>Material Tested</i>	<i>BHA Conc.</i>	<i>No. of Subjects</i>	<i>Procedure</i>	<i>Results</i>	<i>Reference</i>
<i>BHA</i>						
Skin irritation	Face powder	0.2%	20	24-hour patch	PII = 0.03/4; minimal skin irritation	317
Skin irritation	Blusher	0.2%	20	24-hour patch	PII = 0.05/4; minimal skin irritation	318
Skin irritation	Eye shadow	0.2%	20	24-hour patch	PII = 0.15/4; minimal skin irritation	319
Skin irritation	Suntan preparation	0.1%	20	24-hour patch	PII = 0/4; no skin irritation	320
Skin irritation	Bubble Bath	0.1%	19	24-hour patch	PII = 0.95/4; minimal/mild skin irritation	321
Skin irritation and sensitization	Shave cream	0.01%	50	Eight 12-hour induction patches; 2-week rest, one 24-hour challenge patch	During induction phase, reactions ranged from no irritation to slight or well-defined/moderate skin erythema; one slight erythema reaction observed at challenge	322
Skin irritation and sensitization	Shave cream	0.01%	54	Eight 12-hour induction patches; 2-week rest, one challenge patch	During induction phase, reactions ranged from no irritation to slight or well-defined/moderate skin erythema; challenge patch negative	323
Skin irritation and sensitization	Shave cream	0.01%	57	Eight 12-hour induction patches; 2-week rest, one 24-hour challenge patch	During induction phase, reactions ranged from no erythema to severe erythema and edema; no to moderate erythema observed in some individuals at challenge	324
Skin irritation and sensitization	Skin lightener (full strength and 50% aq. dilution)	0.01 to 0.02% (see text)	90	Nine 24-hour induction patches; 4-week rest, one 24-hour challenge patch	During induction phase, 68/90 subjects showed minimal to mild irritation, whereas 22/90 exhibited no skin reaction; 22/90 and 8/90 showed minimal to mild erythema at the 24- and 48-hour challenge readings, respectively	325
Skin irritation and sensitization	Cream	0.2%	108	Draize (1959): Nine 24-hour induction patches; 2-week rest, one 24-hour challenge patch	"Essentially nonirritating" and "no evidence of sensitization"	326

TABLE 7. (Continued.)

Type of Study	Material Tested	BHA Conc.	No. of Subjects	Procedure	Results	Reference
<i>BHA (con't.)</i>						
Skin irritation and sensitization	Skin freshener	0.05%	104	Ten 48-hour induction patches; 10-day rest, one 48-hour challenge patch	No observed reactions to induction or challenge patches	327
Skin sensitization	Liquid makeup	0.01%	26	Repeated insult maximization test (see text)	No reaction observed at challenge on test sites receiving no SLS pre-treatment; investigator concluded "no evidence of contact sensitization"	328
Cumulative skin irritation	Liquid makeup	0.01%	10	Phillips et al. <sup>(331)</sup> : 23-hour patches applied for 21 consecutive days; product allowed to evaporate prior to skin application	Composite total score = 148/630; slight skin irritation	329
Cumulative skin irritation	Polish remover	0.01%	12	Phillips et al. <sup>(331)</sup> : 23-hour patches applied for 21 consecutive days	Composite total score = 26/756; "essentially nonirritating"	330
Cumulative skin irritation	3 different skin creams (A, B, and C)	0.02%	10	Phillips et al. <sup>(331)</sup> : each product applied under 23-hour occluded (o) and semi-occluded (s) patches for 21 consecutive days	Composite total score: Cream A: 547/630(o), 338/630(s); Cream B: 411/630(o), 208/630(s); Cream C: 227/630(o), 200/630(s)	332
Cumulative skin irritation	3 different cosmetic pastes	0.01%	10	Phillips et al. <sup>(331)</sup> : 23-hour patches applied for 21 consecutive days	Skin irritation scores of 46, 40, and 15 were reported for the 3 products, respectively (max. score not specified). Scores of 0 and 644 reported for baby oil (low irritation standard) and deodorant con-	333

Controlled use/skin irritation	Eye makeup	0.1%	51	Haynes and Estrin <sup>(334)</sup> : Product applied in eye area for four weeks under normal use conditions	concentrate (high irritation standard), respectively. Products were considered "essentially nonirritating" to very slightly irritating No observed skin irritation	335,336
Skin irritation, sensitization, and photosensitization	Cosmetic paste	0.01%	45	Draize-Shelanski repeat insult with UV exposure (see text)	One "doubtful" skin irritation reaction observed following the second of 10 closed induction patches; a similar reaction was noted in another subject following the eighth closed induction patch. No reactions observed following any of the 48-hour open or closed challenge patches, nor after UV exposure	337
Skin irritation, sensitization, and photosensitization	Polish remover	0.01%	51	Draize-Shelanski repeat insult with UV exposure; product allowed to evaporate prior to skin application (see text)	A "weak," nonvesicular skin reaction observed in one subject following the second of 10 closed induction patches, and in two other subjects after the sixth closed induction patch. No reactions observed following any of the open or closed 48-hour challenge patches, nor after UV exposure	338
Skin irritation, sensitization, and photosensitization	Cosmetic paste	0.01%	110	Schwartz-Peck prophetic patch with UV exposure (see text)	One individual showed a "weak," nonvesicular skin reaction to the first of two closed patches; no reactions observed following open patches or after UV exposure	339
Skin irritation, sensitization, and photosensitization	Polish remover	0.01%	101	Schwartz-Peck prophetic patch with UV exposure; product allowed to evaporate prior to skin application (see text)	No evidence of skin irritation, sensitization, or photosensitization	340

TABLE 7. (Continued.)

Type of Study	Material Tested	BHA Conc.	No. of Subjects	Procedure	Results	Reference
<i>BHA (con't.)</i>						
Skin irritation, sensitization, and photosensitization	Eye makeup	0.1%	728	Schwartz and Peck <sup>(341)</sup> with UV exposure (see text)	Two "weak," nonvesicular reactions observed after the first of two closed patches, whereas four similar reactions were observed following the second closed patch. No other skin reactions were noted following either closed or open patches, nor after UV exposure. Product considered "nonirritating," "nonsensitizing" and "nonphotosensitizing"	342
Skin irritation, sensitization, and photosensitization	Eye makeup	0.1%	353	Shelanski and Shelanski <sup>(342)</sup> with UV exposure (see text)	"Weak," nonvesicular reactions observed in some subjects after the first 8 of 10 closed induction patches; several subjects exhibited "strong" edematous and/or vesicular skin reactions following the sixth and seventh closed induction patches. Three "weak" nonvesicular reactions observed following the closed challenge patch. Exposure to UV radiation resulted in single "weak" nonvesicular reactions following the first closed induction patch and following the closed challenge patch. No reactions observed to open patches. Product considered "nonirritating," "nonsensitizing" and "nonphotosensitizing"	342
UV exposure	BHA in 50% anhydrous alcohol	100 mg/ml	25	Test material applied to forearm; treated skin areas exposed to UV light for 3 "minimal erythema doses"	BHA conferred moderate skin protection against UV radiation	344

eight induction patches. After a 2-week nontreatment period, one 24-hour challenge patch was applied to each subject and graded at 24, 48, and 72 hours. Skin reactions throughout the induction phase to two of the products ranged from no irritation to slight or well-defined/moderate erythema. Reactions to these same two products at each of the challenge readings were all negative (no erythema), with the exception of one slight erythema reaction to one product, which occurred at the 24-hour 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 observed at the 24- and the 48-hour challenge, whereas no skin erythema to slight skin erythema were noted at the 72-hour challenge. As the severity of skin reactions decreased over the challenge period, so did the number of reactions.<sup>(322-324)</sup> Challenge reactions such as these are not uncommon for skin irritants and skin-fatiguing agents, and they should not be considered evidence of sensitization potential.<sup>(336)</sup>

The skin irritation and sensitization potential of a skin lightener containing 0.02 percent BHA were evaluated in a repeated insult patch test. Eighty-six women and four men between the ages of 18 and 70 were selected for study. A 24-hour occlusive patch containing approximately 0.1 ml of the test material was applied to the same test site on the upper back of each subject every Monday, Wednesday, and Friday for 3 consecutive weeks. A total of nine induction patches was applied. A single 24-hour challenge patch was applied to a fresh site on each subject in Week 7 of the study. The first two induction patches contained the full-strength product; however, subsequent induction and challenge patches contained a 50 percent aqueous dilution of the product (or 0.01 percent BHA: 0.5 × 0.02 percent BHA). The product was diluted after the first two induction patches "to eliminate the irritation properties." During the induction phase, 68/90 subjects showed minimal to mild skin irritation to one or more patches, whereas 22/90 exhibited no skin reactions. Twenty-four and 48 hours after removal of the challenge patch, 22/90 and 8/90 individuals, respectively, showed minimal to mild skin erythema; the remaining subjects demonstrated no skin reactions. The pattern and character of the skin responses indicated an irritation potential for the product under these test conditions. However, the investigator concluded that "Within the limits imposed by the sample size and the test procedure itself. . . , the skin lightener. . . did *not* exhibit any potential for inducing allergic sensitization."<sup>(325)</sup>

One hundred eight adult panelists (70 females, 38 males) were tested to determine their skin response to a cream containing 0.2 percent BHA. The procedure used was a modification of the repeated insult patch test described by Draize.<sup>(345)</sup> The procedure called for a 24-hour occlusive patch to the upper arm every other day for 3 weeks (nine induction applications), a 2-week rest, followed by a 24-hour occlusive challenge patch. The product was "essentially nonirritating" and caused "no evidence of sensitization."<sup>(326)</sup>

A repeated insult patch test was conducted with a skin freshener containing 0.05 percent BHA on 104 subjects. Panelists ranged in age from 18 to 65. The test material was applied to the backs of each subject under an occlusive dressing and held in place for 48 hours. Upon removal of the patch, test sites were graded for skin irritation. An identical patch was then applied to the same site and the procedure repeated for a total of 10 induction applications. After a 10-day nontreatment period, a challenge patch containing the skin freshener was applied to

a new site on the back of each subject. The challenge patch remained in place for 48 hours, and the test sites were graded 15 minutes and 24 hours after patch removal. None of the panelists showed a positive reaction during the induction or challenge phases. However, 11 of the 104 panelists were absent for one or more induction patches, and 13 were absent for the challenge patch.<sup>(327)</sup>

A repeated insult maximization test was conducted on 26 persons with a liquid makeup containing 0.01 percent BHA. The volar aspect of the forearm of each person was pretreated for 24 hours with an aqueous solution containing 5 percent sodium lauryl sulfate (SLS). The liquid makeup was subsequently applied to the SLS-pretreated site under an occlusive patch for "five alternate day 48-hour periods." Following a 10- to 14-day nontreatment period, two 48-hour occluded challenge patches containing the product were applied to fresh sites on the back of each individual. One challenge site was pretreated with a 30-minute occluded application of 2 percent aqueous SLS; the other challenge site received no SLS pretreatment. Skin reactions were graded 48 and 72 hours after each challenge application. One subject showed a reaction at the 48-hour challenge grading on the SLS-pretreated site, whereas seven showed positive reactions on SLS-pretreated sites by the 72-hour evaluation. The severity of these challenge reactions was unspecified. No reactions were observed at challenge on test sites receiving no SLS pretreatment. The investigator concluded that there was "no evidence of contact sensitization."<sup>(328)</sup>

Four separate studies were conducted to evaluate the cumulative skin irritant properties of various cosmetic formulations containing BHA.<sup>(329,330,332,333)</sup> A modification of the procedure of Phillips et al.<sup>(331)</sup> was employed in each of the irritancy tests. In all four studies, the material was applied to the back of each panelist under a 23-hour patch. Daily reapplications of the test sample were made to the same test site for 21 consecutive days. Skin reactions were scored on a scale of 0 (no irritation) to 7 (strong reaction spreading beyond test site). However, the test material was not reapplied to an individual if a score of 3 (erythema and papules) or greater was observed. The composite total score of 10 female panelists tested with a liquid makeup\* containing 0.01 percent BHA was 148 out of a maximum possible value of 630 (21 days × 10 subjects × maximum score of 3); this score was indicative of slight skin irritation. The same panelists were also tested for 21 days with a baby oil and a deodorant concentrate in order to obtain low and high irritation references. Panel scores for the two materials were 8/630 and 589/630, respectively.<sup>(329)</sup> In a second study, a polish remover containing 0.01 percent BHA was found to be "essentially nonirritating." The composite total score for the 12 adult subjects (10 women, 2 men) tested was 26 out of a maximum possible score of 756 (21 days × 12 subjects × maximum score of 3). Scores of 12/756 and 696/756 were reported for baby oil (low-irritation reference) and deodorant concentrate (high-irritation reference), respectively.<sup>(330)</sup> In the third 21-day cumulative irritation test, three different skin creams each containing 0.02 percent BHA were tested on 10 panelists (9 women, 1 man) under both occluded (o) and semioccluded (s) conditions. Baby oil was used as a low-irritation reference; no high-irritation standard was used. The following composite total scores were reported: cream A: 547/630 (o), 338/630 (s); cream B: 411/630 (o), 208/630 (s);

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\* Patches containing the liquid makeup (0.4 ml) were allowed to stand at least 30 minutes prior to skin application to allow for evaporation of the solvents.

cream C: 227/630 (o), 200/630 (s); baby oil: 18/630 (o), 28/630 (s). All scores fell within the skin irritation category "possibly mild in normal use," with the exception of scores for both cream A applied by occluded patch and baby oil. Baby oil was considered a "mild material," whereas cream A applied by occluded patch was considered an "experimental cumulative irritant."<sup>(332)</sup> In the fourth 21-day cumulative irritation test, three different cosmetic pastes each containing 0.01 percent BHA were found "essentially nonirritating" to very slightly irritating. The total skin irritation scores for the panel of 10 women tested with each product were 46, 40, and 15; scores of 0 and 644 were reported for baby oil (low-irritation standard) and a deodorant concentrate (high-irritation standard), respectively. The maximum possible irritation score was not reported in this particular study.<sup>(333)</sup>

Test methods described by Haynes and Estrin<sup>(334)</sup> were used to determine the skin-irritating effects of an eye makeup containing 0.1 percent BHA. The product was applied in the eye area under normal use conditions for 4 weeks. No irritation was noted in any of the 51 test subjects.<sup>(335,336)</sup>

A Draize–Shelanski repeat insult procedure and a UV exposure method were used to evaluate a cosmetic paste for primary skin irritation, skin sensitization, and photosensitization on 45 subjects (1 man and 44 women, aged 18 to 58). The test product containing 0.01 percent BHA was applied to both the upper back and the right upper arm of each subject under one open and one closed 48-hour patch, respectively. Induction applications were made every other day for 3½ weeks for a total of 10 insults. After a 14-day rest, one open and one closed challenge patch were applied to each individual for 48 hours. Closed patch sites were irradiated with UV light following removal of the first, fourth, seventh, tenth, and eleventh (challenge) insults. The UV light source (Hanovia Tanette Mark I Lamp) had a wavelength of 360 nm and was held for 1 minute 12 inches from the skin. One "doubtful" reaction was observed on one individual following the second closed induction patch, whereas a similar reaction was observed in a second panelist following the eighth closed induction patch. No other skin reactions to the cosmetic paste were noted.<sup>(337)</sup> A second study was conducted on 51 subjects (4 men and 47 women, aged 23 to 66) to evaluate a polish remover containing 0.01 percent of the antioxidant. Test procedures were similar to those just described for the previous study, with a major difference being that the product was "allowed to evaporate" 5 minutes prior to application of the occlusive patches. A "weak," nonvesicular skin reaction was observed in one person following the second closed induction patch, and in two other people after the sixth closed induction patch. No other reactions to the polish remover were noted following either induction or challenge insults, nor following UV exposure.<sup>(338)</sup>

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 percent BHA. A 48-hour closed patch containing the paste was applied to the upper back of each of 110 subjects (39 males and 71 females, aged 12 to 50). A 48-hour open patch containing the test material was also applied to the skin of the inside right upper arm of each subject. Following a 14-day nontreatment period, a second set of open and closed patches was applied. The second set of patches was removed after 48 hours, and the closed patch site of each subject was exposed to a Hanovia Tanette Mark I UV source. The lamp had a wavelength peak of 360 nm, and was held from the skin at a distance of 12 inches for 1 minute. Light sensitization was graded 48 hours after

UV exposure. Results following open and closed insults and UV exposure were all negative (no skin reactions) with the exception of one person who demonstrated a "weak," nonvesicular skin reaction to the first closed patch.<sup>(339)</sup> No evidence of skin irritation, skin sensitization, or photosensitization was observed in a second study when 101 subjects (12 males and 89 females, aged 12 to 58) were exposed to a polish remover containing 0.01 percent of the antioxidant. The test procedure varied from the previous study only in that the test material was "allowed to evaporate 5 minutes" prior to application of each 48-hour occlusive patch and in that the 48-hour open patches were applied to the volar aspect of the wrist instead of the arm.<sup>(340)</sup>

Seven hundred twenty-eight subjects were tested with an eye makeup preparation to determine the product's ability to induce skin irritation, skin sensitization, and photosensitization. The product containing 0.1 percent BHA was evaluated according to the methods described by Schwartz and Peck.<sup>(341)</sup> Two 48-hour patches containing the test material were applied to each person. One patch was closed and was applied to the skin of the back. The other patch was open and was applied to the arm. After a 10- to 14-day nontreatment period, a second set of 48-hour closed and open patches was applied. Grading of this second insult was followed by UV irradiation of the closed patch sites. Details of the UV exposure were not specified. Two "weak," nonvesicular reactions were observed after the initial closed patch, whereas four similar reactions were observed following the second closed patch. No other skin reactions were noted following either closed or open patches nor after UV exposure. The eye makeup preparation containing 0.1 percent was considered by the investigator to be "nonirritating," "nonsensitizing," and "nonphotosensitizing" under conditions of this test.<sup>(342)</sup>

The same eye makeup preparation was evaluated in a second study for skin irritation, sensitization, and photosensitization. The test procedure used was a modification of the repeated insult method described by Shelanski and Shelanski.<sup>(343)</sup> Both a closed and open patch containing the test material were applied for 24 hours to each of 353 subjects. The patches were then removed and the test sites left untreated for 24 hours. This "cycle of contact and recuperation" was repeated a total of 10 times over a 3½-week period. Two to three weeks after the tenth induction application, each individual received a 48-hour closed and open challenge patch. Closed patch sites were exposed to UV radiation following the first, fourth, seventh, tenth, and challenge applications (details of the UV exposure were not reported). For closed induction patches 1 through 8, the number of persons with "weak," nonvesicular reactions was 3, 1, 2, 6, 3, 2, 2, and 1, respectively. No reactions were observed following closed induction applications 9 and 10. At the sixth and seventh closed induction patch readings, 3 and 2 people, respectively, exhibited "strong" (edematous and/or vesicular) skin reactions. Three people demonstrated "weak," nonvesicular reactions to the closed challenge patch. No reactions to open patches were observed at any reading. Exposure to both the product and UV radiation resulted in a single, "weak," nonvesicular reaction following the first closed induction patch and in a similar reaction following the eleventh (or challenge) closed patch. Under conditions of this test, the investigator considered the product containing 0.1 percent BHA to be "nonirritating," "nonsensitizing," and "nonphotosensitizing."<sup>(342)</sup>

BHA in 50 percent anhydrous alcohol at a level of 100 mg/ml was applied to the volar aspect of the forearms of 25 white volunteers. When the solution dried



(after 15 minutes), the treated areas were exposed to a sunlamp (280 to 370 nm) at a distance of 2 cm "for an exposure of about 3 minimal erythema doses (MEDs)." BHA protection against UV radiation was evaluated 24 hours after sunlamp exposure. Results were graded visually on a scale of 0 (no protection) to 3\* (complete protection). BHA was given a score of 2.12, indicating that it confers moderate skin protection against UV light.<sup>(344)</sup>

## SUMMARY

BHA is a waxy solid consisting chiefly of 3-t-butyl-4-hydroxyanisole (approximately 90 percent) and 2-t-butyl-4-hydroxyanisole (approximately 8 percent). It is manufactured by tert-butylation of methoxyphenol or by methylation of tert-butylhydroquinone. Reported impurities include 4-hydroxyanisole, 1-t-butyl-2,5-dimethoxybenzene, 2,5-di-t-butyl-hydroxyanisole, hydroquinone dimethyl ether, sulfated ash, lead, and arsenic. BHA exhibits antioxidant properties and prevents lipid peroxidation by providing reactive hydrogen atoms to lipid-free radicals. The compound reacts readily with oxidizing agents to yield quinones. Degradation of BHA results from prolonged exposure to sunlight or UV radiation. BHA is often regarded as "hindered phenol" because its reactivity is decreased by the tert-butyl substitution in the ortho position. The reaction between BHA and nitrite under mild acidic conditions yields a nitrophenol. Although its nitrophenol does not demonstrate mutagenicity, it has been suggested that the compound may be metabolically transformed into toxic substances, such as hydroxylamine derivatives.

BHA is used in cosmetic formulations as a chemical preservative and as an antioxidant. In 1981, cosmetic manufacturers and formulators reported to FDA under a voluntary registration program that BHA was an ingredient in 3217 cosmetic products at concentrations generally ranging from  $\leq 0.1$  percent to 1 percent. Several products were purported to contain the antioxidant at levels of  $> 5$  to 10 percent (three products) and  $> 10$  to 25 percent (one product). The greatest number of reported uses of BHA was in lipstick and eye shadow. Cosmetic products containing the ingredient are applied to or have the potential to come in contact with skin, eyes, hair, nails, and vaginal and nasal mucosae. Small amounts of the antioxidant could be ingested from lipstick.

As a GRAS food preservative, BHA may be used at concentrations not to exceed 0.02 percent (w/w) of the total fat or oil content of a particular food item. Federal regulations also allow BHA to be used as an antioxidant in specific foods at levels ranging from 10 ppm to 0.5 percent (5000 ppm). The Joint FAO/WHO Expert Committee on Food Additives suggested that a dietary level not exceeding 0.5 mg/kg of body weight of BHA, BHT, or the sum of both, would be an acceptable daily intake for man. The daily dietary intake of BHA for man is estimated to be 0.05 to 3 mg.

A comprehensive literature base pertaining to the biological activity of BHA exists. The antioxidant was shown to be effective in inhibiting the growth of bacteria, fungi, protozoa, and bacteriophage. Studies with cultured guinea pig melanocytes revealed that BHA is cytotoxic at concentrations of  $5 \times 10^{-3}$ M, but not at  $5 \times 10^{-6}$ M. BHA at 100 ppm was cytotoxic to cultured myocardial and endotheloid cells isolated from neonatal rats. No depigmentation was observed in guinea pigs or mice when the antioxidant was applied to the skin for 1 to 6 and 2 to 4 months, respectively, at concentrations of 0.1 to 1.0 M. Hypopigmentation

of the down of hatched chicks occurred when 25 ppm BHA was injected into eggs prior to incubation; these results suggested that the antioxidant impaired carotenoid metabolism or deposition. Altered behavioral patterns suggestive of changes in concentrations of neurotransmitters were observed in mice reared on diets containing 0.5 percent (w/w) BHA. The newborn offspring of female mice receiving 0.5 percent of the antioxidant in the diet had reductions in exploratory reflex, body weight, and brain cholinesterase activity. Intraperitoneal doses of 5, 50, and 500 mg/kg BHA increased whole-brain concentrations of 5-hydroxyindole acetic acid in mice. However, insignificant changes were observed in whole-brain concentrations of serotonin and norepinephrine. The antioxidant inhibited prostaglandin biosynthesis in both rat renal medulla at concentrations of 1 mM, and in the microsomal fraction of bovine seminal vesicles at concentrations of 3.08 and 6.70  $\mu\text{M}$ . No alteration in the concentrations of pituitary gonadotropic hormone was observed in rats or guinea pigs fed BHA for 6 months at doses of 0.4 mg/kg/day, whereas inhibition of bradykinin activity was observed in isolated uterine muscle of rats and ileal muscle of guinea pigs treated with  $10^{-6}\text{M}$  and  $8 \times 10^{-9}$  mol/L of BHA, respectively. Disorganization of mitochondrial structure and inhibition of cellular respiration were attributed to BHA in studies with guinea pig liver homogenate and with the mitochondrial-lysosomal fraction of rat liver. The ability of the antioxidant to alter biomembrane structure and function was also noted in several studies. It has been suggested that the high electron density associated with the aromatic nucleus of BHA enables the antioxidant to interact with hydrophobic and electrophilic regions of membranes.

BHA given orally or parenterally to mice and rats was shown to inhibit the carcinogenic effects of a broad range of chemical carcinogens under a variety of experimental conditions. The mechanism of the anticarcinogenic effect has not been determined, but may involve (1) alteration of metabolism of the carcinogen 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 of transport, and (4) competitive inhibition. Several studies indicated that the tumor-inhibiting effects of BHA may be altered by dietary factors. BHA has also been shown to inhibit mutagenesis both in vitro and in vivo. Findings suggest that the protective effect of BHA may be due to its ability to inhibit the formation of mutagenic metabolites.

The ability of the antioxidant to affect the activation of a number of mammalian enzymes is well documented. However, whether BHA stimulates or inhibits enzyme activities varies according to test conditions. Quantitative changes in enzymatic activity due to BHA treatment appear to be related to dosage and duration of administration. Induction of drug-metabolizing enzymes by BHA is often accompanied by liver enlargement. This enlargement is an adaptive response. At concentrations at which BHA induces liver hypertrophy there is no evidence of persistent hepatotoxicity.

Both animal and human studies have shown that BHA is absorbed from the gastrointestinal tract and metabolized. In man, BHA is conjugated with glucuronic acid in the liver to form glucuronide. Urinary excretion products consist primarily of glucuronides, with lesser amounts of sulfates and free BHA. No dealkylation or hydroxylation products are detected. In one study, 27 to 77 percent of a single 0.4 to 0.7 mg/kg oral dose of BHA was excreted in the urine as glucuronide within 23 to 38 hours; less than 1 percent of the dose appeared in the urine as ethereal sulfate or as intact BHA.

Although tissue storage may occur with BHA because of its lipid solubility, the amount stored may be quite limited because of rapid metabolism and excretion. Chickens and pigs fed diets containing 0.1 percent BHA for 8 weeks and 4 months, respectively, showed no accumulation of the antioxidant in muscle, liver, kidney, or reserve fat. Similarly, dogs maintained for 1 year on diets containing BHA up to 100 mg/kg as a 50 percent solution in propylene glycol showed no storage of antioxidant in body fat, brain, liver, or kidney. Rats fed 2 to 3 percent BHA for 6 months had only trace amounts of BHA in depot and carcass fat.

Reported acute oral LD<sub>50</sub> values for BHA in rats and mice varied from 2.0 to >5.0 g/kg and 1.1 to 2.0 g/kg, respectively. Cosmetic products formulated with either 0.1 or 0.2 percent of the antioxidant were determined to have oral LD<sub>50</sub>s in rats of >5 g/kg, whereas an eye makeup formulation containing 0.1 percent BHA had a dermal LD<sub>50</sub> in rabbits of >2 g/kg. Formulations with similar concentrations of BHA elicited at most minimal or moderate skin and eye irritation in rabbits. Guinea pig immersion studies with bubble bath products containing 0.1 percent BHA revealed no evidence of systemic toxicity.

A number of subchronic and chronic oral studies with the antioxidant were conducted on a variety of animals, including rats, rabbits, chickens, dogs, guinea pigs, and monkeys. The effect of BHA on such factors as growth, survival, behavior, organ weights, blood counts, blood chemistries, enzymatic activity, electrolyte balance, fat metabolism, hormonal activity, sex cycle, reproduction, and function of the liver, kidney, adrenal, and reproductive glands varied according to test conditions and to dosage and duration of antioxidant administration. One frequent finding was enlargement of the liver and/or increased liver weight. However, these changes were not generally accompanied by persistent hepatotoxicity. Livers of monkeys given BHA for 4 weeks at doses of 500 mg/kg/day exhibited a marked proliferation of smooth endoplasmic reticulum, accumulation of lipid droplets, enlarged nuclei and nucleoli, fragmentation of the nucleolus, and randomly dispersed nucleolar fibrils. The livers of dogs fed BHA at doses of 250 mg/kg/day for 15 months showed parenchymal degeneration, diffuse granulocytic infiltration, marked narrowing of sinusoids, accumulation of biliary pigment and increased hemosiderin storage in Kupffer cells. In other studies, microscopic examination of kidneys of rats fed diets containing up to 1.35 percent BHA for 110 days revealed necrosis, expansion of the renal cavity, and epithelial cell swelling in tubules. Female rats fed a mixture of BHA (10 mg/kg) and propylgallate (20 mg/kg) for 9 months failed to produce progeny when mated with similarly fed males. The antioxidant was lethal to rabbits following oral doses of 1 g for 1 to 7 days; death was attributed to excessive potassium excretion.

No evidence of carcinogenicity was observed when BHA was administered to mice by subcutaneous injection (single 10 mg dose in trioctanoin), by intraperitoneal injection (1.2 or 6.0 g/kg in tricaprylin three times a week for 8 weeks), or by topical application (0.1 or 10 mg in acetone once a week for 323 to 509 days, or 1 mg in acetone twice a week for 30 weeks). No carcinogenesis was demonstrated following dietary administration of BHA to either rats (up to 0.12 percent for 21 to 22 months, or up to 0.1 percent for 2 years) or dogs (up to 250 mg/kg/day for 15 months, or up to 0.3 percent for 1 year).

An increased incidence of forestomach papillomas and squamous cell carcinomas was observed by Ito et al.<sup>(283)</sup> in rats fed BHA at dietary concentrations of 0.24 and 1.07 percent for 2 years. A group of health officials representing four

different nations (Japan, Canada, United Kingdom, United States) recently evaluated the results of this particular study as it relates to human consumption of BHA. Their conclusion, as stated in the "Report of the Principal Participants of the Four Nations,"<sup>(10)</sup> was as follows:

While one of the principals (Japanese representative) stated that human consumption of BHA should be avoided for the present until there is a better understanding of the mechanism by which BHA produces its effect, the consensus among the other principals is that BHA does not appear to act as a classical carcinogen, that a safety factor of thousands of fold exist and that there would be little, if any, risk to human health from delaying any action until such time that there is a better understanding of the mechanism by which BHA produced its effects in the Ito study. Furthermore, there may be health risks inherent in the replacement or removal of BHA from current uses arising from other phenolic antioxidants or from the oxidative products of fats, and that further research is needed to the area of phenolic antioxidants to more precisely identify these risks.

BHA failed to show mutagenic activity against *S. typhimurium* or *S. cerevisiae* when tested in a series of in vitro assays with and without the addition of mammalian metabolic activation preparation. The antioxidant at in vivo doses of 15, 150, and 1500 mg/kg did not induce mutations in a host-mediated assay using *S. typhimurium*. However, significant increases in recombinant frequencies occurred at each of these doses in a host-mediated assay using *S. cerevisiae*. The antioxidant was nonmutagenic in a dominant lethal study and caused no significant aberration of bone marrow chromosomes when given to rats orally at doses of 15, 150, and 1500 mg/kg. In vitro chromosomal aberration tests with human embryonic lung cultures and Chinese hamster fibroblasts were also negative. *D. melanogaster* fed BHA had no increase in sex-linked recessive lethals. 2-Tert-butyl hydroquinone, a degradation product resulting from BHA exposure to UV radiation, was mutagenic in assays with wild and recombinationless strains of *B. subtilis* and wild and rad mutant strains of yeast.

Oral administration of BHA to pregnant rabbits at doses up to 200 mg/kg during gestation caused decreased survival of both dams and fetuses and an increase in the ratio of resorptions to number of implant sites. However, the number of abnormalities observed in either skeletal or soft tissues did not differ significantly from the number occurring spontaneously in sham-treated controls. Additional studies with pregnant rabbits, mice, rats, and hamsters receiving BHA during gestation by a variety of oral dosage regimens revealed no significant embryotoxic or teratogenic effects.

Clinical data for BHA were primarily derived from studies with cosmetic formulations containing 0.01 to 0.2 percent of the antioxidant. Although these formulations were generally nonsensitizing, nonphotosensitizing, and only minimally or mildly irritating, there were reported instances of several products eliciting either sensitization, photoreactions, or severe skin erythema and edema in some individuals. Whether these reactions were attributable to BHA or other ingredients in the formulations was not ascertained. Case reports of patients exhibiting various allergic reactions following ingestion of BHA and contact dermatitis following skin application of BHA-containing products have been cited in the literature. The incidence of contact dermatitis among 548 subjects patch tested

with 2 percent BHA was reported by the North American Contact Dermatitis Group to be 2 percent (11 subjects). Results from one clinical study suggested that BHA in anhydrous alcohol moderately protects exposed skin against UV radiation.

## CONCLUSION

On the basis of the available information presented in this report, the Panel concludes that BHA is safe as a cosmetic ingredient in the present practices of use.

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## BUTYLATED HYDROXYANISOLE (BHA)

A safety assessment of Butylated Hydroxyanisole was published in 1984 with the conclusion that this ingredient is safe as a cosmetic ingredient in the practices of use (Elder 1984). New studies, along with updated information regarding types and concentrations of use, were considered by the CIR Expert Panel. The Panel determined to not reopen this safety assessment.

The name of Butylated Hydroxyanisole as listed in the *International Cosmetic Ingredient Dictionary and Handbook* has been changed to BHA (Pepe et al. 2002).

BHA functions in cosmetics include antioxidant and fragrance ingredient. It was used in 3217 cosmetic products in 1981, with the largest use occurring in lipstick at concentrations of  $\leq 10\%$  (Elder 1984). In 2002, BHA was used in 1224 cosmetic products (FDA 2002), at a maximum use concentration of 0.2% in colognes, toilet waters, and perfumes (CTFA 2003). Table 3 presents the available use information for BHA. The most recent information now constitutes the present use of this ingredient.

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**TABLE 3**  
Historical and current cosmetic product uses and concentrations for BHA

Product category	1981 uses (Elder 1984)	2002 uses (FDA 2002)	1981 concentrations (Elder 1984) %	2003 concentrations (CTFA 2003) %
<b>Baby care</b>				
Lotions, oils, powders, and creams	1	1	>0.1–1	0.0001
<b>Bath</b>				
Oils, tablets, and salts	20	4	≤0.1	0.0004
Bubble baths	7	—	≤0.1	0.00001
Bath soaps and detergents	2	5	≤0.1	0.000004
Other bath	10	3	≤1	0.0001
<b>Eye makeup</b>				
Eyebrow pencil	33	51	≤1	0.0001
Eyeliner	75	399	≤1	0.1
Eye shadow	410	38	≤5	0.002
Eye lotion	2	2	≤0.1	—
Eye makeup remover	11	6	≤0.1	0.02
Mascara	65	18	≤1	0.1
Other eye makeup	39	10	≤1	0.001
<b>Fragrances</b>				
Colognes and toilet waters	97	18	≤1	0.2
Perfumes	62	6	≤1	0.2
Powders	12	2	≤0.1	0.0002
Sachets	21	—	≤0.1	—
Other fragrances	24	10	≤1	0.004
<b>Noncoloring hair care</b>				
Conditioners	8	5	≤0.1	0.0002
Sprays	1	—	—	0.0001
Shampoos	6	—	≤0.1	0.0005
Tonics, dressings, etc.	10	8	≤1	0.02
Wave sets	1	—	—	—
Other noncoloring hair care	—	—	—	0.05
<b>Hair coloring</b>				
Other hair coloring	5	1	≤0.1	—
<b>Makeup</b>				
Blushers	176	26	≤5	0.2
Face powders	98	11	≤1	0.005
Makeup foundations	119	30	≤0.1	0.05
Lipstick	1256	279	≤25	0.2
Makeup bases	64	4	≤1	0.005
Rouges	48	1	≤1	0.04
Makeup fixatives	10	—	≤0.1	—
Other makeup	106	23	≤5	0.05
<b>Nail care</b>				
Basecoats and undercoats	1	3	≤0.1	—
Cuticle softeners	2	2	≤0.1	0.001
Creams and lotions	4	1	≤0.1	—
Polish and enamel	—	—	—	0.06
Polish and enamel remover	1	—	≤0.1	—
Other nail care	2	4	≤0.1	0.004

**TABLE 3**  
Historical and current cosmetic product uses and concentrations for BHA (*Continued*)

Product category	1981 uses (Elder 1984)	2002 uses (FDA 2002)	1981 concentrations (Elder 1984) %	2003 concentrations (CTFA 2003) %
<b>Oral hygiene</b>				
Dentifrices	—	—	—	0.01
<b>Personal hygiene</b>				
Underarm deodorants	1	1	≤0.1	0.002
Other personal hygiene	2	4	≤1	0.002
<b>Shaving</b>				
Aftershave lotions	11	2	≤1	0.006
Preshave lotions	3	—	—	—
Shaving cream	8	10	≤0.1	0.0003
Shaving soap	1	—	>0.1–1	—
Other shaving	3	—	≤1	0.0003
<b>Skin care</b>				
Cleansing creams, lotions, etc.	51	23	≤1	0.05
Face and neck skin care	77*	15	≤1*	0.1
Body and hand skin care	—	72	—	0.1
Hormone skin care**	1	**	—	**
Foot powders and sprays	—	1	—	0.004
Moisturizers	111	51	≤1	0.06
Night skin care	30	26	≤1	0.04
Paste masks/mud packs	6	3	≤1	0.004
Skin lighteners**	11	**	≤0.1	**
Skin fresheners	6	2	≤0.1	—
Wrinkle smoothers**	6	**	≤0.1	**
Other skin care	42	30	≤1	0.03
<b>Suntan</b>				
Suntan gels, creams, and liquids	27	7	≤1	0.1
Indoor tanning	2	1	≤0.1	—
Other suntan	9	5	≤0.1	—
<b>Total uses/ranges for BHA</b>	<b>3217</b>	<b>1224</b>	<b>≤0.1–25</b>	<b>0.000004-0.2</b>

\*These categories were combined, but now are listed separately.

\*\*No longer listed as product categories.

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## BUTYLENE GLYCOL, HEXYLENE GLYCOL, ETHOXYDIGLYCOL, AND DIPROPYLENE GLYCOL

A safety assessment was published in 1985 with the conclusion that these ingredients are safe as presently used in cosmetics (Elder 1985). New studies, along with updated information regarding types and concentrations of use, were considered by the CIR Expert Panel. The Panel determined to not reopen this safety assessment.

**Butylene Glycol** was reported to be used in 165 cosmetic preparations in 1981, with the greatest use occurring in mascara, and at concentrations that ranged from less than 0.14% to greater than 50% (Elder 1985). In 2002, industry reports to FDA indicated that Butylene Glycol was used in 813 preparations (FDA 2002). An industry survey of use concentrations in