# Amended Safety Assessment of BHA as Used in Cosmetics

Status: Draft Amended Report for Panel Review
Release Date: March 4, 2024
Panel Meeting Date: March 28 – 29, 2024

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, Senior Scientific Analyst/Writer, CIR.


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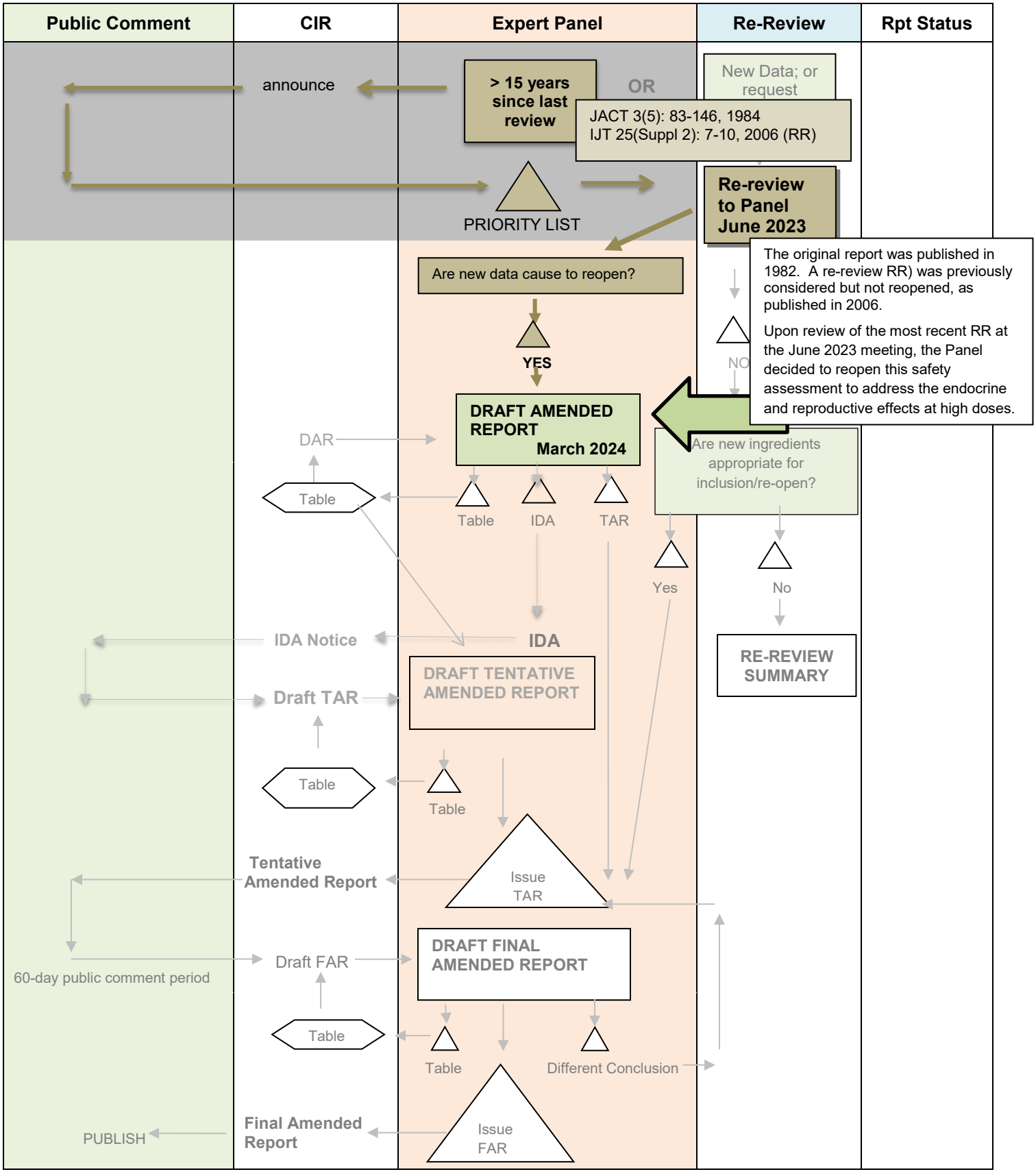
# RE-REVIEW FLOW CHART

**INGREDIENT/FAMILY** BHA

**MEETING** March 2024



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**Memorandum**

To: CIR Expert Panel Members and Liaisons
From: Preethi S. Raj, M.Sc.
Senior Scientific Analyst/Writer, CIR
Date: March 4, 2024
Subject: Amended Safety Assessment of BHA as Used in Cosmetics

Enclosed is a Draft Amended Report on the Safety of Assessment of BHA as Used in Cosmetics. (It is identified as *report_BHA_032024* in the pdf document). The Expert Panel for Cosmetic Ingredient Safety (Panel) first published a review of the safety of Butylated Hydroxyanisole (since renamed as BHA) in 1984 (*originalreport_BHA_032024*). 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). During initial re-review deliberations in September 2003, the Panel evaluated data from an unpublished document (*RRdata_BHA_032024)* and reaffirmed the 1984 conclusion, as published in 2006 (*RRSum2006_BHA_032024*). At its June 2023 meeting, the Panel decided to reopen this safety assessment to evaluate potential endocrine and reproductive effects of BHA at high doses and to provide an updated assessment of this ingredient.

Since the last review, reported use categories have not changed significantly and concentrations of use have remained constant over time. According to 2023 VCRP data, BHA has 70 reported uses; at the time this ingredient was last considered for re-review, 1224 uses were reported. In 2023, the maximum reported concentration of use for BHA was 0.15% in other manicuring preparations, while BHA was reported to be used at up to 0.2% in several product formulations (cologne and toilet waters, perfumes, blushers, and lipstick) in 2003. An exposure assessment of BHA in nineteen different cosmetic product use categories has been prepared by Dr. Jinqiu Zhu and is included in the report for the Panel's consideration.

Additional supporting documents for this report package include: a flow chart (*flow_BHA_032024*), report history (*history_BHA_032024*), search strategy (*search_BHA_032024*), data profile (*dataprofile_BHA_032024*), the minutes from past meetings at which BHA was discussed (*originalminutes_BHA_032024*), concentration of use data (*data_BHA_032024*), and transcripts from the previous meeting at which the rereview of BHA was discussed (*transcripts_BHA_032024*).

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Amended Report. However, if additional data are required, the Panel should be prepared to identify those needs an issue an Insufficient Data Announcement.

# BHA History

**1984**

- The Expert Panel for Cosmetic Ingredient Safety (Panel) first published a Final Report with the conclusion that that Butylated Hydroxyanisole (since renamed as BHA) is safe as a cosmetic ingredient in the present practices of use

**2006**

- A re-review of BHA was published, re-affirming the 1984 conclusion

**June 2023**

- An extensive search of the available published literature since 1999 was conducted in accordance with CIR Procedures regarding re-review of these ingredients after ~ 15 years. The Panel determined that this safety assessment should be reopened to evaluate potential endocrine and reproductive effects of BHA at high doses, and to provide an updated assessment of the ingredient

**March 2024**

- **A Draft Amended Report is being presented to the Panel for review.**

| BHA Data Profile* – March 28 - 29, 2024 Panel Meeting – Writer, Preethi Raj | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Use | | | | Toxico-kinetics | | | Acute Tox | | | Repeated Dose Tox | | | DART | | Genotox | | Carci | | Dermal Irritation | | | Dermal Sensitization | | | | Ocular Irritation | | Clinical Studies | |
| | New Rpt | Old Rpt | Method of Mfg | Impurities | log P/log $K_{ow}$ | Dermal Penetration | ADME | Dermal | Oral | Inhalation | Dermal | Oral | Inhalation | Dermal | Oral | In Vitro | In Vivo | Dermal | Oral | In Vitro | Animal | Human | In Vitro | Animal | Human | Phototoxicity | In Vitro | Animal | Retrospective/ Multicenter | Case Reports |
| BHA | X | O | O | O | X | | OX | O | O | | | OX | | | OX | OX | O | O | O | | O | O | | | O | O | | O | | O |

* "X" indicates that new data were available in this category for the ingredient; "O" indicates that data from the original assessment were available

# [BHA]

| Ingredient | CAS # | PubMed | FDA | HPVIS | NIOSH | NTIS | NTP | FEMA | EU | ECHA | ECETOC | SIDS | SCCS | AICIS | FAO | WHO | Web |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BHA | 25013-16-5 | ✓ | ✓ | ✓ | ✓* | NR | ✓ | ✓ | ✓ | ✓* | NR | NR | NR | ✓ | ✓ | ✓ | ✓ |

✓ -data available
✓*-relevant data is unavailable
NR – not reported

## Botanical and/or Fragrance Websites (if applicable)

| Ingredient | CAS # | Dr. Duke's | Taxonomy | GRIN | Sigma-Aldrich | AHPA | AGRICOLA | IFRA | RIFM |
|---|---|---|---|---|---|---|---|---|---|
| BHA | 25013-16-5 | NR | NA | NA | NA | NA | NA | NR | NR |

## Search Strategy – most recently performed on 02/04/2024

General: butyl hydroxyanisole sensitization; butyl hydroxyanisole non animal testing methods; butyl hydroxyanisole dpra; butyl hydroxyanisole ppra; butyl hydroxyanisole keratinosens; butylated hydroxyanisole carcinogenicity

PubMed: (BHA) OR (25013-16-5) AND (2001:2024[pdat]) – 1553 hits/12 useful

((((((((((((((((((((BHA) OR (25013-16-5) ) OR (butylated hydroxyanisole)) OR ((1,1-Dimethylethyl)-4-Methoxyphenol)) OR (BHA (HCPA))) OR (Butylhydroxyanisol)) OR (Butylated Hydroxyanisole)) OR (Phenol, (1,1-Dimethylethyl)-4-Methoxy-)) OR (Eastman Tenox BHA)) OR (OriStar BHA)) OR (Uantox BHA)) OR (Chebiretiether)) OR (Chebiretipeptide)) OR (Eastman Tenox 4)) OR (Eastman Tenox 4B)) OR (Eastman Tenox R)) OR (TagraPlus E1)) OR (Uantox 3)) OR (Uantox HW-4)) OR (Uantox W-1)) OR (Vyox)) AND (toxicity) AND (2001:2024[pdat]) – 309, 531 hits/10 useful

((((((((((((((((((((BHA) OR (25013-16-5) ) OR (butylated hydroxyanisole)) OR ((1,1-Dimethylethyl)-4-Methoxyphenol)) OR (BHA (HCPA))) OR (Butylhydroxyanisol)) OR (Butylated Hydroxyanisole)) OR (Phenol, (1,1-Dimethylethyl)-4-Methoxy-)) OR (Eastman Tenox BHA)) OR (OriStar BHA)) OR (Uantox BHA)) OR (Chebiretiether)) OR (Chebiretipeptide)) OR (Eastman Tenox 4)) OR (Eastman Tenox 4B)) OR (Eastman Tenox R)) OR (TagraPlus E1)) OR (Uantox 3)) OR (Uantox HW-4)) OR (Uantox W-1)) OR (Vyox)) AND (toxicity) AND (endocrine) AND (2001:2024[pdat]) – 10, 302 hits/6 useful

(((("BHA"[All Fields] OR "25013 16 5"[EC/RN Number] OR ("butylated hydroxyanisole"[MeSH Terms] OR ("butylated"[All Fields] AND "hydroxyanisole"[All Fields]) OR

# LINKS

## Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
  - appropriate qualifiers are used as necessary
  - search results are reviewed to identify relevant documents
- Connected Papers - https://www.connectedpapers.com/

## Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA Cosmetics page - https://www.fda.gov/cosmetics
- eCFR (Code of Federal Regulations) - https://www.ecfr.gov/
- FDA search databases: https://www.fda.gov/industry/fda-basics-industry/search-databases
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
- SCOGS database: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database
- Inventory of Food Contact Substances Listed in 21 CFR: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives
- Drug Approvals and Database: https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases
- FDA Orange Book: https://www.fda.gov/drugs/drug-approvals-and-databases/approved-drug-products-therapeutic-equivalence-evaluations-orange-book
- OTC Monographs - https://dps.fda.gov/omuf
- Inactive Ingredients Approved For Drugs: https://www.accessdata.fda.gov/scripts/cder/iig/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program ) - http://ntp.niehs.nih.gov/
- EUR-Lex - https://eur-lex.europa.eu/homepage.html
- Scientific Committees (SCCS, etc) opinions: https://health.ec.europa.eu/scientific-committees_en https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en
- ECHA (European Chemicals Agency – REACH dossiers) – https://echa.europa.eu/
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- EFSA (European Food Safety Authority) - https://www.efsa.europa.eu/en
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) IRIS library - https://apps.who.int/iris/
- a general Google and Google Scholar search should be performed for additional background information, to identify references that are available, and for other general information - www.google.com https://scholar.google.com/

## Botanical Websites, if applicable

- Dr. Duke's - https://phytochem.nal.usda.gov/
- Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
- GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
- Sigma Aldrich plant profiler- http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html
- American Herbal Products Association Botanical Safety Handbook (2nd Edition; 2013) - http://abc.herbalgram.org/site/DocServer/AHPABotanicalSafety_FMexcerpt.pdf?docID=4601
- National Agricultural Library NAL Catalog (AGRICOLA) https://agricola.nal.usda.gov/
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

## Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – https://ifrafragrance.org/
- Research Institute for Fragrance Materials (RIFM) - https://www.rifm.org/#gsc.tab=0 http://fragrancematerialsafetyresource.elsevier.com/
-

## JUNE 2023 PANEL MEETING – INITIAL REVIEW/RE-REVIEW

### Belsito Team – June 12, 2023

**DR. BELSITO:** Okay. BHA. Another Women's Voice for the Earth fav. Okay, we've got a Wave 2 and a Wave 3. Wave 2 is largely Women's Voice for the Earth. Wave 3, I think was PCPC comments.

**DR. SNYDER:** Right.

**DR. BELSITO:** -- on that material.

**DR. SNYDER:** Two and three.

**DR. BELSITO:** Yeah.

**DR. SNYDER:** A lot of new data.

**DR. BELSITO:** A lot of new data. I think we need to reopen this. I'm not sure that our conclusion will likely change, but we need to address all the endocrine and reports at high dose. We need to look at the Zhang paper that Women's Voice for the Earth refers to. We need to review the data in the California database that they state reports higher concentrations that we're being told in this report. So, we need to reopen it.

**DR. SNYDER:** Agree.

**DR. RETTIE:** Yep.

**DR. BELSITO:** And I mean, I'm sure we can get the California database, right? That has to be publicly available?

**DR. ZHU:** It's available.

**DR. BELSITO:** Yeah.

**DR. ZHU:** That's is an information variance. Some discontinued. So that's mostly like VCRP, you can get to the most outdated data. You have to go to check one ingredient (inaudible).

**DR. BELSITO:** Okay. Well, we still should get it so that we're aware of what's out there.

**DR. KLAASSEN:** I guess what I was thinking when I read over this, the original document was written, well, last century, I guess. And there's a lot of new data and I just wonder if it shouldn't be written from scratch.

**DR. BELSITO:** Well, we will essentially write it from scratch. The stuff in the old report will be italicized. All the new stuff will come in and there's lots of new stuff. So, it'll basically end up looking like a new document.

**DR. KLAASSEN:** Okay.

**DR. BELSITO:** Okay.

**DR. KLAASSEN:** But the categories are different. I don't know how you would combine the two, but --

**DR. BELSITO:** Well, you look at what was actually in the category.

**DR. KLAASSEN:** You put it in the new category?

**DR. BELSITO:** Yes. You don't carry it over as the old category. You carry over the data but not in the new category heading.

### Cohen Team – June 12, 2023

**DR. COHEN**: BHA, Butylated Hydroxyanisole. A safety assessment was published in 1984, with a safe is used. And the Panel reconsidered it in 2006, with a reaffirmation. It's been at least 15 years. A number of studies evaluating endocrine activity, reproductive and development effects of BHA have been found.

There was a Dutch cohort examining the association between the dietary intake of BHA and stomach cancer risk. We have 2023 VCRP, with BHA used in 70 products. And the max use has remained fairly close. Comments from the group?

**DR. BERGFELD**: Did you want to add the use data that was presented by the Women's Voices of the Earth, which is higher.

**DR. COHEN**: Yes. I was going to open up the Wave 2 after.

**DR. ROSS**: Well, just based on the data we have, the use data is lower. The new data material, not in the original, I guess the most notable is the endocrine studies. It's whether or not you deem that sufficient to reopen the dossier or not based on that endocrine data, I think.

**DR. TILTON**: Now is it based on --

**DR. ROSS**: We had the WVE request -- or let's finish -- the CIR analysis showed products for the highest concentration withdrawn. And also there was an acceptable daily intake study. So, I think for me it revolves around -- whether we reopen or not revolves around the endocrine studies.

**DR. COHEN**: Which is a very hot topic.

**DR. BERGFELD**: Hot, hot topic.

**DR. ROSS**: Yeah.

**DR. BERGFELD**: I think we have to reopen.

**DR. COHEN**: Tom?

**DR. SLAGA**: There's lots of new data. Most of it's the same, but there are some differences. And it actually even goes into some isomers of BHA. But when you look at it all, it's really the same conclusion, even though there are some hot items that are listed. But in the past we have eliminated those because it really wasn't that relevant at the doses used, especially the endocrine.

So, you know, I went back and forth about do not reopen or reopen and deal with the sensitivity of some of those issues again. But it's going to put us in the same condition, same conclusion, so I kind of lean towards do not reopen, even though there's a lot of data.

**DR. COHEN**: Lisa? Do you have anything different?

**DR. SLAGA**: We'll probably get criticized if we did that.

**DR. TILTON**: Susan.

**DR. COHEN**: Huh?

**DR. ANSELL**: Susan.

**DR. COHEN**: I'm sorry, what did I say?

**DR. ANSELL**: Lisa.

**DR. COHEN**: Oh, yeah, that was a flashback. Sorry, Susan.

**DR. TILTON**: I agree with a lot of what's been said. In terms of the reproductive studies that were included, I mean, I noted that most of them were related to changes in gene expression, not specifically linked to any specific adverse outcome. There were reproductive studies with some effects, but they were mostly not just dependent, and inconclusive.

So, under the information that was provided to us with the significant reduction in use and no change in concentration, I was not a fan of reopening. But like everyone else, with the new information on the increase in the number of formulations that are being used, and even the max use concentration, and the fact that this includes some new data on new isomer, if we feel like we need to explore additional data -- I don't think that the data that was provided here is really striking in terms of concern about reproductive effects. But that hasn't really been evaluated in the context of these different products that were suggested or those concentrations.

**DR. COHEN**: I actually came to the same conclusions as Tom, which is I don't know if any of the scientific information in front of me was going to change the result. But so, for that reason I was going to not reopen. The problem is this report is 40 years old, right? And no matter how good our re-review summary is, it is not going to provide a reasonably updated report on a chemical that people really do have an interest in.

This comes up. So is another reason to reopen the fact that the old report, despite having a similar conclusion, has a body of data that is too old to rely on as a report? People look for our reports when they're doing their scientific research, when they're writing papers. These are references. And this BHA reference is 40 years old.

**DR. BERGFELD**: But when you update it -- even if you said not reopen, you would update all the references. But do you think the search may not be in depth enough?

**DR. COHEN**: It's going to be a paragraph, right, or half of page. I don't know. Your thought?

**DR. BERGFELD**: I felt we ought to reopen for a lot of reasons.

**DR. ANSELL**: So, not dramatically different. I just point out that it is not an inconsequential amount of work to reopen.

**DR. COHEN**: Yeah.

**DR. ANSELL**: And so, I'm not sure that the data alone is enough. There's a lot of new data, but it's not going to result in a significantly different conclusion. The California database is very difficult to rely on, for a number of reasons. So, you know,

based on just the amount of work for a material, which is unlikely to have any significant change, we recommended not to reopen.

**DR. COHEN**: Wilma, what are you thinking because you opened the can about reopening?

**DR. BERGFELD**: Yeah, I appreciate all of what you say, Jay. I just see that these endocrine disruption type of phenomena are very important to the public right now. And that we do have an increase in concentrations. We have these isomers, so we may have something just a little bit different here. It would be nice to update that and say it was negative.

So, I guess I'm standing on the side of the public here and I would reopen. Also the Women's Voices of the Earth has requested it because of the increase in concentrations and uses right now.

**DR. COHEN**: Does the staff believe the concentration of use has gone up? Because your reply may have suggested that it might not have.

**DR. ROSS**: Something about discontinuation of products at the highest concentration

**DR. COHEN**: If they were even at that concentration, right?

**DR. HELDRETH**: Right?

**DR. ANSELL**: Yeah. That would be our position. I mean, just looking at the function, there would be no reason to have that much. But we do know that the database is difficult to rely on.

**DR. COHEN**: Yeah.

**DR. ANSELL**: Based on that, cadmium is still widely used.

**DR. ROSS**: Yeah. The two products at high concentration has been discontinued as of 10/22. I mean, that's based on concentration. In my original comments, I think it just depends how you value this new data on the endocrine studies, and what weight you place on that.

**DR. ANSELL**: And what we often see when we delve into it is that it was a mixture, which is used at 15 percent. But the BHA is used at the efficacious amount in the tens of the percent. But it gets reported as 15.

**DR. COHEN**: You mean because the raw material in there was 15?

**DR. ANSELL**: Yeah.

**DR. COHEN**: I don't know how reliable --

**DR. BERGFELD**: But in the formulation it was decreased. In the formulation it was less, much less.

**DR. ANSELL**: Right. It's used at the appropriate concentration for BHA in a product, which is at 15 percent in the formulation.

**DR. BERGFELD**: Yeah. So that could be translated into discussion plus translated in the document. We've done that before.

**DR. ANSELL**: Yeah.

**DR. BERGFELD**: The active ingredient is this concentration. Yeah.

**DR. ROSS**: I had this as a plus/minus. And based on the number of uses, it was a minus. Based on the new endocrine studies it was a plus. So, you know, it's one of those.

**DR. COHEN**: As a plus that would change your outcome, or as a plus to include the data in the report?

**DR. ROSS**: No, plus to reopening.

**DR. TILTON**: For the changed conclusion?

**DR. COHEN**: For what? For what purpose? To include the discussion of the endocrine data? Would that change your outcome of safe as used in the concentrations reported here?

**DR. ROSS**: Well, we would have to review all the endocrine data, because that's new.

**DR. HELDRETH**: I'd like to add a little note.

**DR. COHEN**: Yeah, please do.

**DR. HELDRETH**: The philosophy behind the re-review process, as I understood it -- first I'm hearing it a lot, is has the use changed? You know, if suddenly it was a new type of exposure, the concentration was more, well, we definitely want to reopen and go after it. But I think there are other reasons to do a re-review.

Has the science changed? Has the perspective of the science changed? I mean, when we first reviewed this in 1984, the panel didn't have an opinion on endocrine activators and so forth, in that time. Since then, we've come out with the Panel's own viewpoint and we have document on the CIR website that talks about it.

So, I mean, if incorporating that information into the Panel's review and, like David said, updating the document that's available for dermatologists or other researchers to know about it, that's also an equally reasonable reason --

**DR. BERGFELD**: To reopen.

**DR. HELDRETH**: -- to reopen.

**DR. COHEN**: Yeah. I think you've just maybe tilted the scale a little bit on that.

**DR. HELDRETH**: Uh-oh.

**DR. COHEN**: No, no, no, no, no. In a positive way.

**DR. ROSS**: Yeah.

**DR. COHEN**: And I don't think an updated report on BHA is going to just be a dermatology interest. This is going to be endocrinology, oncology, epidemiology. It's a 40-year old report. No matter how you put gift wrap on it with a re-review summary, it's never going to be a new report.

**DR. ROSS**: I'm okay.

**DR. COHEN**: Okay. It'll be interesting though, this will also be another conversation because I think this has a -- Don's presenting this tomorrow. I wouldn't be surprised if it's a re-review. Okay.

**MS. RAJ**: So, am I hearing that you wanted to reopen?

**DR. COHEN**: Yeah, I think we're going to reopen. I think we all had the same -- all struggled on it.

**Full Panel – June 13, 2023**

**DR. BELSITO**: This is another ingredient that we received comments from Women's Voices for the Earth. We looked at all of the data. We felt we needed to reopen this. Our conclusion likely will not change, but we do need to address the endocrine and reproductive reports at high doses, and would like information from the California database about products out there that may have higher concentration of use than we've been told.

**DR. COHEN**: Second.

**DR. BERGFELD**: Any additional needs or requests for this ingredient?

**DR. COHEN**: Don covered everything that we had.

**DR. BERGFELD**: All right. Call the question, all those in favor of reopening BHA? Okay, unanimous. Then, Dr. Cohen you're next on the Octoxynols.

## DECEMBER 1982 PANEL MEETING: INITIAL REVIEW

Butylated Hydroxyanisole (BHA)

Dr. Schroeter expressed concern regarding the depigmentation potential of this ingredient and recommended contacting Dr. Pathak to obtain results of his depigmentation study on p-hydroxyanisole for possible relevance to BHA. The Panel also discussed the Japanese study on the incidence of tumors of the forestomach of rats. Dr. Elder advised the Panel that CIR had already contacted Dr. Pathak and asked for the test data quoted in his published abstract; however, Dr. Pathak had not responded. CIR would again ask for the information in order that the Panel could review it at the April meeting. Dr. McEwen noted that the existing report quoted a published paper which indicated that BHA was not a depigmenter. A motion to delay further consideration of this report until a response is received from Dr. Pathak was unanimously accepted.

## APRIL 1983 PANEL MEETING: SECOND REVIEW/DRAFT TENTATIVE REPORT

Dr. Schroeter's Team had previously requested Dr. Pathak provide the Panel with a copy of his data on BHA depigmentation; no response was obtained. It was agreed that because the abstract contradicts other published data on this subject, the Panel disregard the abstract of his study for use in this report, except to reference it in the minutes as follows:

Pathak, M.A., Farinelli, W.A., and Fitzpatrick, T.B.: Cutaneous depigmentation by certain antioxidants, azelaic acid and dopa derivatives. Clin. Res. 27(2):244 (abstract), 1979.

The Panel reviewed the "Report of the Principal Participants of the Four Nations (Canada, Japan, U.K., and U.S.) on the Evaluation of the Safety of BHA." There was concurrence among the Panel members that the report is worthwhile and that it puts Dr. Ito's study into its proper perspective. They also concurred that a ban on BHA -- based on only one positive carcinogenic study in light of the numerous negative studies -- is not justified at this time.

It was agreed that the following paragraph from the Four-Nation Report which summarizes the consensus of the representatives should be quoted in the Minutes:

"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 in the area of phenolic antioxidants to more precisely identify these risks."

This statement will also be incorporated into the Summary of the BHA Tentative Report.

The following conclusion of the report was unanimously approved: "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."

Subject to minor revisions, the document will be announced as a Tentative Report for a 90-day comment period.

[unable to locate minutes for when the Final Report was issued]

## SEPTEMBER 2003: FIRST RE-REVIEW

Dr. Marks stated that a CIR Final Report with the following conclusion was published in 1984: 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. He added that the new data included in the re-review document do not warrant a decision by the Panel to reopen the Final Report.

The Panel voted unanimously in favor of not reopening the Final Report on BHA, and agreed that a discussion is not needed.

# Amended Safety Assessment of BHA as Used in Cosmetics

Status: Draft Amended Report for Panel Review
Release Date: March 4, 2024
Panel Meeting Date: March 28 – 29, 2024

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**,** Senior Scientific Analyst/Writer, CIR.


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## ABBREVIATIONS

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

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

## INTRODUCTION

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

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

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

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

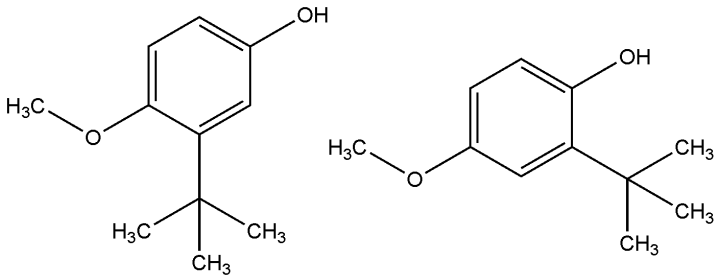
Summarized excerpts from the initial report on BHA and an unpublished document containing data considered by the Panel during the initial re-review process in September 2003 are included in this document, as indicated by *italicized* text. (This information is not included in the tables or the summary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

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

## CHEMISTRY

### Definition and Structure

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




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

Figure 1. BHA

### Chemical Properties

*BHA (MW = 180.2 g/mol) is a white or slightly yellow, waxy solid having an aromatic odor.[2] BHA exhibits antioxidant properties and acts synergistically with acids, butylated hydroxytoluene, propyl gallate, hydroquinone, methionine, lecithin, and thiodipropionic acid in protecting lipids autooxidation. The use of a synergistic combination will result in a greater stability than can be obtained by using the equivalent quality of either antioxidant alone. A BHA sample of > 99% purity has a protein-binding capacity of 4680 mmol/mole protein and a hydrophobic bonding ability (expressed as the difference in log partition coefficients of BHA and phenol) of 1.88.* BHA also has a log $K_{ow}$ of 3.5 and a water solubility of 0.213 g/l at 25 °C.[4] Other chemical properties of BHA can be found in Table 1.

### Method of Manufacture

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

### Impurities

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

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

## USE

### Cosmetic

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

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

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

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

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

### Non-Cosmetic

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

antioxidant in resinous and polymeric coatings (21CFR175.300), as a defoaming agent (21CFR176.210), and as a component of polyethylene film (21CFR179.45).

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

## TOXICOKINETIC STUDIES

### Dermal Absorption

**In Vitro**

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

### Absorption, Distribution, Metabolism, and Excretion

**In Vitro**

*The binding of BHA to human albumin was studied in vitro.[2] The percent binding of BHA as a function of total concentration in the "protein compartment" ranged from a minimum of 68% to a maximum exceeding 90%. It was noted that the ingestion of a 2 mg/kg dose of BHA yields a "maximum concentration" of approximately 26 µg/ml, assuming that all the BHA initially remains in the "blood compartment." Thus, a high degree of protein binding can be assumed during normal ingestion of BHA.*

**Animal**

**Oral**

*Multiple studies in rats have established that BHA is absorbed from the gastrointestinal tract and metabolized.[2] Groups of 4 or 8 male and female Sprague-Dawley rats were administered a single oral dose of BHA at up to 400 mg/kg, in various isomeric forms. Urinary excretion of BHA glucuronide and ethereal sulfate during 5 d post-dosing accounted for 61 – 82 and 11 – 16% of the recovered dose, respectively; 5% of the dose was excreted unchanged. For single oral doses of 2, 10, 25, 50, 100 mg/kg BHA overall recovery in the urine was 81 – 100%, with a slight increase in the excretion of unchanged BHA at the lower doses. A single oral dose of 400 mg/kg 2-BHA was excreted in 5 d; 72% of the dose was excreted as ethereal sulfate. For 3-BHA, 57 – 71% of a 400 mg/kg dose was excreted as glucuronide in 5 d. Several successive daily doses of 500 mg/kg BHA and of 3-BHA decreased the percent recovery of conjugates and the proportion of excreted ethereal sulfate. Repeated doses of 500 mg/kg 2-BHA resulted in a considerable increase in glucuronide and a decrease in ethereal sulfate conjugation. The researchers suggested that 4-O-conjugation, O-demethylation, hydroxylation of the phenyl ring, and sidechain oxidation (-CH2, -CH2·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. Ninety percent of a single dose of 80 – 100 mg BHA given orally to rats was excreted in the urine as glucuronide conjugate (71%), ethereal sulfate (14%), and unconjugated phenol (5%).*

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

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

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

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

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

**Human**

**Oral**

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

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

Male subjects (number not specified) received a single, oral dose of 50 mg 3-BHA (vehicle not specified).[13] The test substance was excreted in the urine as 27 – 77% glucuronide and urinary metabolites. In another study, subjects (sex and number not specified) received a single, oral dose of 0.5 mg/kg 3-BHA. Most of the test substance was recovered in the urine and feces (95%). 3-BHA was excreted in its conjugated form in the urine and as conjugated *tert*-butyl hydroquinone in the feces. No free 3-BHA was found in the urine or feces.

## TOXICOLOGICAL STUDIES

### Acute Toxicity Studies

**Dermal**

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

**Oral**

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

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

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

## Short-Term Toxicity Studies

### Oral

*Four male Sprague-Dawley rats were given 500 mg/kg/d BHA via gavage for 6 d.[2] Total daily sodium excretion in the urine of treated animals was less than expected from food intake on days 2 -6, possibly owing to interference with renal prostaglandin synthesis. Male rats were given 500 mg/kg/d BHA in the diet for 1, 2, 4, or 6 d; p-aminohippurate (PAH) accumulation was reduced in the kidney after 1 dose of BHA. Increases in liver weight following the second dose were accompanied by increases in PAH serum to slice ratio, but these increases approached normal levels after 6 doses. Male and female SPF Carworth rats ( n = 4 – 12/sex/group) were given 0, 50, 100, 200 or 500 mg/kg/d BHA for 7 d, via gavage. No changes in liver fat were noted; however, significant increases in liver weights were observed in females in the 50, 200, and 500 mg/kg/d groups and in males from the 100, 200, and 500 mg/kg/d groups. No changes were observed with respect to fat metabolism, weight gain, liver glycogen, cholesterol, phospholipids, or concentration and iodine number of liver fat, in rats that were administered 4 mg/kg/d BHA in the diet for 30 to 35 d. Male and female Norway hooded rats (6/sex/group) received 0, 0.1, 0.2, 0.3, 0.4, or 0.5% BHA) in the diet for 6 wk. Increased levels of total serum cholesterol were noted in animals fed 0.1% BHA. Increasing dietary concentrations of BHA were associated with increased absolute lipid content and liver weight; no histologic changes were attributable to treatment. No changes were observed with respect to growth, composition of hepatic polyunsaturated fatty acids, serum levels of sodium, total liver lipid concentration, or total and esterified cholesterol levels of liver and adrenals. Eight rats were given 400 mg/kg/d BHA in the diet for 8 wk; besides a decrease in phagocytic activity of leukocytes, no change in various blood characteristics was observed. Rats consumed 500 mg/kg/d BHA for 82 d or 600 mg/kg/d BHA for 68 d, both in the diet. Lag in weight gain and reduced blood catalase and peroxidase activities and increases in liver weights and body fat content were observed; however, upon necropsy, no pathologic differences were noted between treated and control animals. Female Carworth Farm SPF rats (n = 4 - 5) were administered 500 mg/kg/d BHAfor 84 d, via gavage. Significant increases in liver weights and liver protein were observed; activities of hepatic hexobarbital oxidase, nitroanisole demethylase, codeine demethylase, and aminopyrine demethylase were not affected. Researchers thought it appropriate to disregard hyperfunctional liver enlargement in assessing the acceptability of BHA as a food ingredient. Male and female weanling Carworth SPF rats (n = 24/sex) were administered a diet containing 0.1% BHA (for up to 16 wk. Toward the end of the 16-wk period, there was a decrease in food consumption and retardation of growth in males. Increases in relative liver weight, and in a few instances, adrenal weights, occurred predominantly in females. No significant changes in hepatic glucose-6-phosphatase occurred in either sex after 16 wk; however, a decrease in this enzyme's activity was noted in females after 4 wk. No histopathologic evidence of damage to the liver was observed.*

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

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

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

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

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

## Subchronic Toxicity Studies

### Oral

*Rats (n = 20/group) were given 0. 0.05, 0.15, 0.45, or 1.35% BHA in the diet for 110 d.[2] Males in each treatment group exhibited increased liver weight but normal weights for brain, pituitary, thyroid, thymus, heart, testis, prostate, spleen, and adrenals; male rats fed 0.05% BHA showed increased lung and kidney weights. Female animals demonstrated increased thymus weight at 0.05%, increased thyroid weight at 0.15, 0.45, and 1.35%, and increased liver weight at all dose levels. Brain, pituitary, heart, lung, spleen, adrenal, kidney, uterus, and ovary weights of female rats were comparable in all treated female groups, compared to controls. Microscopic examination of the kidneys of several animals of both sexes revealed necrosis (all dose levels), expansion of the renal cavity (all dose levels), and epithelial swelling in the tubules (0.15, 0.45, and 1.35%).*

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

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

## Chronic Toxicity Studies

### Oral

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

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

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

## DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

### Oral

*No discernible effect on nidation or on maternal or fetal survival were observed in female CD-1 mice that received up to 225 mg/kg/d BHA, via gavage, from day 6 to 15 of gestation.[2] The number of abnormalities seen in either soft or skeletal tissues of the test groups was similar to that occurring spontaneously in sham-treated controls. BHA was administered via oral intubation, in arachis oil, to ICI SPF female mice (500 mg/kg/d for 7 wk before mating and until day 18 of gestation; abnormalities were considered spontaneous, since they were also observed in untreated groups. Concurrently, BHA was orally administered to Tuck albino rats (750 mg/kg/d from day 1 to 20 of gestation), Tuck albino and Benger hooded rats (750 mg/kg/d for 70 d before mating and throughout gestation), Tuck and Carworth SPF albino rats (single administration of 1000 mg/kg on day 9, 11, or 13 of gestation), and Porton albino rats (500 mg/kg/d for 7 wk before mating and throughout gestation). All doses retarded growth of weanling albino female rats and produced weight loss in adults. However, no significant embryotoxic or teratogenic effects were seen in any strain of either species (albino or hooded). The abnormalities were considered spontaneous, since they were also observed in untreated groups. Negative teratogenic results were also reported for Wistar albino rats that received up to 200 mg/kg/d BHA from day 6 through 15 of gestation and for hamsters that received 120 mg/kg/d BHA from day 6 through 10 of gestation. No deleterious effects on reproduction in terms of 21-d litter weights or numbers of pups born and weaned were observed in 80 Norway hooded rats fed up to 0.5% BHA in the diet for 8 mo. Neither rats nor guinea pigs, that received 4 mg/kg/d BHA in the diet for 6 mo, showed impaired function of the reproductive glands. There was also no observed adverse effects in either animal with respect to sex cycle phases, activity of gonadotropic pituitary hormone glands, or histology of endocrine glands.*

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

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

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

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

The effect of BHA upon embryoid body development was evaluated in P19C5 stem cells.[16] The lowest-observed-adverse-effect-level (LOAEL) for BHA affecting embryoid body morphogenesis was 400 µM; BHA influenced the expression of developmental genes in a temporal and gene-specific manner. Groups of male and female Sprague-Dawley rats (12/sex/group) were given 0, 10, 100, or 500 mg/kg BHA, in corn oil, via gavage, in a generational developmental and reproductive toxicity study.[17] Weights of liver, adrenal and thyroid glands were increased, mating rate was decreased, and cohabitation during conception was longer in the $F_0$ (parental) generation. Body weights were significantly reduced in the

500 mg/kg group at postnatal day 21, liver and adrenal gland weights were increased, while weights of the spleen, vagina, testes, and ventral prostate were decreased in the $F_1$ (filial) generation rats exposed to 100 or 500 mg/kg BHA for 13 wk. Additionally, reduced velocity of sperm motion and number, lowered serum levels of thyroxine and testosterone, slightly shortened estrous cycle length, and effects in the follicular epithelial cells of the thyroid were observed in $F_1$ rats exposed to 500 mg/kg BHA. In another reproductive toxicity study, male and female rats received 0, 110, 220, or 420 mg/kg/d BHA, in the diet; the NOAEL for maternal toxicity was determined to be 420 mg/kg/d for.[15] For offspring toxicity, a NOAEL of 220 mg/kg/d BHA and a LOAEL of 420 mg/kg/d BHA was established, based on the reduced weight of progeny during lactation and increasing peri-weaning mortality.

## GENOTOXICITY STUDIES

### In Vitro

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

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

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

### In Vivo

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

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

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

## CARCINOGENICITY STUDIES

### Dermal

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

### Oral

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

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

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

### Tumor Promotion

### Dermal

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

### Oral

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

### Other Routes

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

## ANTI-CARCINOGENICITY STUDIES

*The BHA-mediated protection of rodents against the neoplastic effects of carcinogens is considered to be nonspecific with respect to the chemical nature of the carcinogen, the route of carcinogen administration, or the site of tumor formation.[2] Proposed mechanisms of the anti-carcinogenic effect of BHA include (1) alteration of the carcinogen metabolism by decreased activation, increased detoxification, or both, (2) scavenging of active molecular species of carcinogens to prevent their reaching critical target sites in the cell, (3) alteration of permeability or transport, and/or (4) competitive inhibition. It has been suggested that BHA may be metabolized by mixed function oxidases in the same manner as the carcinogens, thereby allowing BHA metabolites to compete with ultimate carcinogens for the binding with cellular macromolecules.*

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

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

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

Female ACI rats ($n \geq 10$/group) were treated with cholesterol (controls), BHA, 17β-estradiol ($E_2$), or a combination of 17β-estradiol and BHA for 7, 15, 120, or 240 d.[18] Mammary tumor development was monitored twice weekly during treatment. Rats in the $E_2$ and the combined $E_2$ + BHA groups were implanted subcutaneously with pellets of 3 mg $E_2$ combined with 17 mg cholesterol. Control and BHA only groups had pellets containing only cholesterol implanted. $E_2$ and control group animals were fed a purified phytoestrogen-free diet, while $E_2$ + BHA and BHA only groups were fed a diet containing 0.7% BHA (w/w). Upon completion of treatment, animals were killed and mammary tissues/tumors as well as liver, uterus, kidney, lung, brain, and ovary tissues were obtained for histopathological analyses. Total 8-isoprostane $F_{2\alpha}$ levels, a marker of oxidant stress, and the activity of other antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) were quantified in the mammary and liver tissue. Neither cholesterol control nor BHA-treated groups developed mammary tumors. Tumor latency was significantly increased in the $E_2$ + BHA group, which exhibited a 24% incidence after 8 mo of treatment compared to the $E_2$ only group which exhibited the first palpable tumor after 128 d of treatment, and had an 82% tumor incidence after 8 mo. Histopathological examination of mammary tissue from the control and BHA groups revealed normal structure. No differences in tumor morphology between $E_2$ or $E_2$ + BHA-treated animals were observed, with the exception of more invasiveness in the mammary tumors resulting from $E_2$-treatment. No evidence of tumor or dysplasia was observed in non-target organs. Mammary tissue from rats treated with $E_2$ + BHA for 120 and 240 d displayed significantly lower levels of 8-isoprostane $F_{2\alpha}$ (suggesting inhibition of $E_2$-mediated increases). Superoxide dismutase activity was significantly reduced in rats exposed to $E_2$ + BHA for 240 d, compared to the $E_2$-treated group. No significant differences were observed in the activities of glutathione peroxidase and catalase enzymes in mammary tissue, or in superoxide dismutase, glutathione peroxidase, and catalase in the liver tissues of $E_2$ + BHA and BHA only groups.

## OTHER RELEVANT STUDIES

### Endocrine Effects

In a study evaluating the in vitro effects of BHA on testicular cell function, immature mouse Leydig cells and Sertoli cells were grown in serum-free medium to 60% confluence prior to treatment with 0, 10, 20, 50, 75, or 100 µM BHA for 24 h.[19] Cytosolic and mitochondrial calcium ion concentration levels were disrupted and endoplasmic reticulum stress signaling pathways were induced in Leydig and Sertoli cells, relative to vehicle-treated controls. The relative mRNA expression of genes involved in the biosynthesis of steroids were significantly downregulated after treatment with BHA in both cell lines.

The steroidogenic effects of BHA were evaluated in immature rat Leydig cells.[20] Cells were isolated from 35-d old rats and cultured with 50 µM BHA for 3 h, in combination with 22R-OH-cholesterol, prenenolene, progesterone, androstenedione, testosterone, or dihydrotestosterone, and the concentrations of 5α-androstenediol and testosterone were measured in the media. Real-time polymerase chain reaction (q-PCR) was used to measure the mRNA expression levels of the following genes: *Lhcgr* (luteinizing hormone/chorionic gonadotropin receptor), *Scarb1* (scavenger receptor class B type 1), *Star* (steroidogenic acute regulatory protein), *Cyp11a1* (cholesterol side-chain cleavage enzyme P450scc), *Hsd3b1* (3β-hydroxysteroid dehydrogenase/λ(4) isomerase type 1), *Srd5a1* (3-oxo-5α-steroid-4-dehydrogenase), and *Akr1c14* (aldo-keto reductase family 1 member C1). The testis microsomes were prepared to detect the direct action of BHA on HSD3B1, 17α-hydroxylase (CYP17A1), and 17β-hydroxysteroid dehydroxygenase-3 activities. BHA significantly inhibited androgen production, rat testis CYP17A1 and HSD3B1 activity, and expression levels of *Hsd17b3* and *Srd5a1*, leading to lower production of androgen in Leydig cells.

BHA was evaluated for its anti-androgenic activity in a reporter gene assay and an androgen receptor antagonist test using the human MDA-kb2 breast cancer cell line.[21] For the androgen receptor agonist test, cells were exposed simultaneously to a constant concentration of 1000 pM 5-α-dihydrotestosterone (DHT), a strong androgen receptor agonist, with increasing concentrations of each test material (0, 0.15, 0.5, 1.5, 5, 15, 30, 50 150, or 300 mM), in DMSO When tested in the presence of 1000 pM DHT, BHA exhibited significantly anti-androgenic activity in a concentration-dependent manner. At the highest concentration (300 μM), BHA completely inhibited DHT-induced luminescence

In another luciferase assay, the anti-androgenic effects of BHA was evaluated in transfected human PC-3 prostate carcinoma cells.[22] Cells were incubated for 18 h with each test material (0, 0.1, 1, or 10 μM) in the presence or absence of 50 pM DHT. BHA did not inhibit androgenic activity in the absence of DHT, but did antagonize DHT-induced activation of the androgen receptor such that at the 10 μM test concentration the DHT-induced effect was inhibited by at least 50%. Cell viability was not affected by treatment with BHA. The half maximal effective concentration ($EC_{50}$) for BHA to inhibit DHT-mediated activation in the luciferase assay was 7.6 μM; BHA was considered a partial androgen antagonist.

The estrogenic and anti-estrogenic effects of BHA were evaluated in an luciferase assay using T47D-Kbluc breast cancer cells and in an estrogen-dependent proliferation assay, using MCF-7 breast cancer cells.[23] In the luciferase assay, cells were treated with increasing concentrations (0, 0.15, 0.5, 1.5, 5, 15, 30, 50, 150, or 300 mM), in DMSO, for 24 h; estradiol was used as a positive control, at concentrations of 30 pM and 10 pM, in each assay. Anti-estrogenic effects were evaluated in the proliferation assay; cells were incubated in the presence of 10 pM estradiol. BHA exhibited a higher potency of estrogenic effects in the luciferase assay than in the proliferation assay.

The anti-androgen-, glucocorticoid-, and thyroid hormone-like activities of BHA were evaluated in multiple luciferase assays, using MDA-kb2 and GH3.TRE-Luc reporter cell lines.[24] BHA exhibited anti-glucocorticoid-like and anti-androgen activity

The androgenic activity of BHA was evaluated in a Hersherberger assay.[25] 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, *levator ani plus bulbocarvernosus* muscle, and Cowper's gland were not affected by treatment with BHA alone. The relative testosterone propionate-stimulated ventral prostate weight was significantly increased by the 250 mg/kg BHA dose, but the absolute and formalin-fixed weight was not significantly changed.

The anti-estrogenic activity of BHA was evaluated in immature female rats.[25] Twenty-day-old, female Sprague-Dawley rats (11/group) received subcutaneous injections of 0, 50, 100, 250, or 500 mg/kg BHA, in corn oil, once per day for 3 consecutive days. To investigate anti-estrogenic activity, the injection of 50 or 500 mg/kg BHA was followed by an injection of 2 μg/kg $E_2$ (in corn oil), administered within 10 min for 3 d. Six 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 $E_2$-stimulated increase of relative and absolute uterine and vaginal weight. BHA treatment did not affect uterine epithelial height, either alone or with $E_2$ treatment.

The potential endocrine disruptive effects of BHA were evaluated in female Wistar rats.[26] Immature (17-21 d) female Wistar rats (10/group) received 300 mg/kg bw BHA, in sunflower oil, via gavage for 3 d. A 20 μg/kg dose of $E_2$, in sunflower oil was administered via subcutaneous injection to positive controls. Rats were weighed and killed using diethyl ether; the genital tract, liver, spleen, and kidneys were removed, weighed, and prepared for histopathological analyses. Except for negative and positive controls, no significant changes in body weight were observed in treated groups. Rats treated with BHA had minimal histopathological changes in the uterus, with no changes in the cervix and vagina. BHA, butylated hydroxytoluene, and propyl gallate had the ability to decrease the relative uterine weight; propyl gallate had the most severe effect. Though not statistically significant, an increase in endometrial epithelium cell height was observed with BHA treatment, suggesting a possible estrogenic effect.

**Immunomodulatory Effects**

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

BHA was found to promote phagocytosis of macrophages from peripheral blood mononuclear cells; this effect was not observed in macrophages from the peritoneal cavity. BHA-treatment did not alter the cytotoxicity of NK cells.

### Hepatic Effects

*Induction of drug-metabolizing enzymes by BHA is often accompanied by liver enlargement.[2] The Select Committee on GRAS Substances reported that the enlargement of the liver and stimulation of microsomal drug-metabolizing enzymes observed with BHA are produced by at least 200 compounds of extremely diverse pharmacologic activities. Referring to a number of studies, the Committee suggested that liver enlargement (also referred to as "work hypertrophy," "physiological overworking," or "hyperfunctional enlargement") is an adaptive response. It was noted that at levels at which BHA induces liver hypertrophy, there is no evidence of persistent hepatotoxicity.*

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

### Hormonal Effects

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

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

### Effect on Melanocytes

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

### Effects on Human Astrocytes

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

## DERMAL IRRITATION AND SENSITIZATION STUDIES

### Irritation

#### Animal

*A suntan preparation containing 0.1% BHA and an eye shadow and a face powder each containing 0.2% BHA were evaluated for their skin irritation potential in 3 separate tests.[2] In each study, the test formulation (0.1 ml) was applied for 24 h under occlusion to the shaved skin of 9 albino rabbits. Test sites were graded for irritation on a scale of 0 – 4 at 48 and 72 h after the initial reading. The primary irritation indices were 0.46 (suntan preparation), 0.4 (eye shadow), and 0.11 (face powder) indicating that the formulations were "minimal" or "slight" skin irritants. An eye makeup preparation*

*containing 0.1% BHA was evaluated in another skin irritation study, according to procedures specified in 16 CFR 1500.41. A 24-h, occlusive application of the test material (0.5 g) was made to the intact and abraded skin of 6 albino rabbits. Test sites were graded for irritation upon patch removal and 48 h after this first reading; the primary irritation index was 2.75, indicating that the product was a moderate skin irritant.*

*Two guinea pig immersion tests were conducted to evaluate percutaneous toxicity and the dermal irritation potential of 2 different bubble bath formulations, each containing 0.1% BHA. Guinea pigs in each study had their abdominal hair clipped and were immersed up to their axillae in a 0.5% aqueous solution of the product. Twelve animals (6 animals/ product) were exposed to an actual BHA concentration of 0.005% (0.005 x 0.1%) 4 h/d for 3 consecutive days. Skin reactions on the abdomen were graded on a scale of 10 (normal skin) to 1 (moribund due to skin injuries) 48 h following the last exposure. The average irritation indices for the 2 formulations were 7.9 and 5, indicating mild (moderate scaling, no loss of skin elasticity), and moderate (cracking and fissuring, considerable loss in skin elasticity) skin irritation, respectively. No evidence of systemic toxicity was observed in either test.*

**Human**

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

**Sensitization**

**Human**

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

**Photosensitization**

**Human**

*A Draize-Shelanski HRIPT and an ultraviolet (UV) exposure method was used to evaluate a cosmetic paste containing 0.1% BHA for primary skin irritation, skin sensitization, and photosensitization in 45 subjects.[2] A pair of 1 open and 1 closed 48-h induction patches were applied every other day for 3.5 wk for a total of 10 induction applications. After a 14-d rest, 1 open and 1 closed patch were applied for a 48 h-challenge application. Closed patch sites were irradiated with UV*

*light following removal of the first, fourth, seventh, tenth, and eleventh (challenge) insults. One "doubtful" reaction was observed in 1 individual following the second closed induction patch, and a similar reaction was observed in another subject following the eighth closed induction patch. No other reactions were observed following any of the 48-h open or closed challenge patches, nor after UV exposure. In a similar study testing a polish remover containing 0.01% BHA, a "weak" non-vesicular skin reaction was observed in 1 subject following the second of 10 closed induction patches and in 2 other subjects after the sixth closed induction patch. No other reactions to the polish remover were noted during induction or challenge insults or following UV exposure.*

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

## OCULAR IRRITATION STUDIES

### Animal

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

## CLINICAL STUDIES

### Case Reports

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

*developed vesicles on their hands and lips within 12 h of being orally challenged with BHA. Daily oral administration of 5 or 10 mg BHA for 4 d caused a flare-up of skin dermatitis in BHA-sensitive individuals. A 32-yr-old patient reacted with generalized urticaria in a double-blind study following ingestion of BHA. The patient had persistent cryoglobulinemia that did not change with "challenge" and normal baseline histamine concentrations that elevated with challenge to BHA.*

*Two patients had contact dermatitis reactions to pharmaceutical-grade BHA (2%) in Timodine® cream.[12] However, the 2 patients had negative reactions to analytical-grade BHA (2%) in patch tests. The researchers could not explain the discrepancy. Seven patients who had allergic contact dermatitis to some cosmetics and toiletries were found to be sensitive to 1% BHA. Two elderly patients developed eczema after applying a topical cream treatment for psoriasis. Patch tests showed positive reactions with 2% BHA, an ingredient in the topical cream.*

**Cohort Study on Dietary Exposure to BHA**

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

## EXPOSURE ASSESSMENT

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

## SUMMARY

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

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

In a toxicokinetics study, rats were fed 0.12% 3-BHA in the diet for 21 mo. The test substance was absorbed from the gut via passive diffusion; no evidence of tissue storage was observed in the animals. An unspecified number of male subjects received a single oral dose of 50 mg 3-BHA; the test substance was excreted in the urine as 27 – 77% glucuronide and urinary metabolites. In another study, most of a single, oral dose of 0.5 mg/kg 3-BHA administered to human subjects was recovered in the urine and feces (95%). 3-BHA was excreted in its conjugated form in the urine and as conjugated *tert*-butyl hydroquinone in the feces. No free BHA was found in the urine or feces.

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

The LOAEL for BHA affecting embryoid body morphogenesis in P19C5 stem cells was 400 µM; BHA influenced the expression of developmental genes in a temporal and gene-specific manner. Groups of male and female Sprague-Dawley rats (12/sex/group) were given up to 500 mg/kg BHA, in corn oil, via gavage, in a generational developmental and reproductive toxicity study. The weights of liver, adrenal and thyroid glands were increased, mating rate was decreased, and cohabitation during conception was longer in the $F_0$ generation exposed to 500 mg/kg BHA. $F_1$ rats exposed to 500 mg/kg BHA exhibited significant reductions in the body weights at postnatal day 21, increased liver and adrenal gland weights, decreased spleen, vagina, testes, and ventral prostate weights, reduced velocity of sperm motion and number, lowered serum levels of thyroxine and testosterone, slightly shortened estrous cycle length, and effects in the follicular epithelial cells of the thyroid. In another developmental toxicity study, in which rats were dosed with up to 420 mg/kg/d BHA in the diet, the NOAEL for maternal toxicity was determined to be 420 mg/kg/d. Based on reduced weight of progeny during lactation and increasing peri-weaning mortality, a NOAEL of 220 mg/kg/d BHA and a LOAEL of 420 mg/kg/d BHA were established for offspring toxicity.

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

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

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

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

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

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

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

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

## PREVIOUS DISCUSSIONS

### Discussion from Original Report Published in 1984

*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 $LD_{50}$ values for BHA in rats varied from 2000 to > 5000 mg/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 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 non-sensitizing, non-photosensitizing, and only minimally or mildly irritating. It is concluded that BHA is safe as a cosmetic ingredient in the present practices of use.*

### Discussion from Re-Review Summary Published in 2006

*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. 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 Butyl Hydroxyanisole as listed in the International Cosmetic Ingredient Dictionary and Handbook has been changed to BHA. BHA functions in cosmetics as an antioxidant and fragrance ingredient. It was used in 3217 cosmetic products in 1981, with the largest use occurring in lipstick at concentrations of ≤ 10%. In 2002, BHA was used in 1224 cosmetic products, at a maximum use concentration of 0.2% in colognes, toilet waters, and perfumes.*

## DISCUSSION

To be developed.

## CONCLUSION

To be determined.

# TABLES

**Table 1. Chemical properties of BHA**

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

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

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

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

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

NR – not reported

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

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

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

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

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

**Table 3. Developmental and reproductive and toxicity studies**

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

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

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

| Product Category/Type of cosmetics exposure | Daily Exposure by Product Category* (mg/d) | Maximum Concentration of Use | Daily Exposure Based on the Highest Use Concentration (mg/d) | Note |
|---|---|---|---|---|
| **Eyebrow pencils** | 20 | 0.05% | 0.01 | Exposure amount of eye shadow applied |
| **Eyeliners** | 5 | 0.05% | 0.0025 | |
| **Eye shadows** | 20 | 0.000086-0.05% | 0.01 | |
| **Mascaras** | 25 | 0.03% | 0.0075 | |
| **Other fragrance preparations** | 1500# | 0.001% | 0.015 | Exposure amount of eau de toilette spray applied |
| **Hair conditioners** | 40 | 0.0084% | 0.00336 | |
| **Hair sprays Aerosol** | 5000 γ | 0.00000004% | 0.000002 | |
| **Shampoos (noncoloring)** | 110 | 0.0024% | 0.00264 | |
| **Face powders** | 85† | 0.05% | 0.0425 | |
| **Foundations** | 510 | 0.02% | 0.102 | |
| **Lipstick** | 60 | 0.05% | 0.03 | |
| **Other manicuring preparations** | 300 # | 0.15% | 0.45 | Exposure amount of nail polish applied |
| **Dentifrices** | 138 | 0.00045% | 0.000621 | Exposure amount of toothpaste applied |
| **Bath soaps and detergents** | 49.8 | 0.0006-0.0022% | 0.0011 | Exposure amount of bath oil, salts, etc. applied |
| **Deodorants**<br>**Not spray**<br>**Aerosol** | <br>1500<br>6540 | <br>0.00076%<br>0.000051% | <br>0.0114<br>0.003335 | |
| **Skin cleansing (cold creams, cleansing lotions, liquids and pads)** | 190 | 0.00025% | 0.000475 | Exposure amount of shower gel applied |
| **Face and neck products Not spray** | 1540 | 0.00013-0.013% | 0.20 | Exposure amount of face cream/lotion applied |
| **Body and hand products Not spray** | 7820 | 0.0021% | 0.0164 | Exposure amount of body lotion applied |
| **Night products Not spray** | 308 # | 0.00001% | 0.000031 | Exposure amount of face mask applied |

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

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

γ Exposure amount is provided by CTFA (currently known as PCPC) habits and practices data[32]

# Exposure amount is provided by Vermeer Cosmolife[33]

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

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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 $LD_{50}$ 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.**

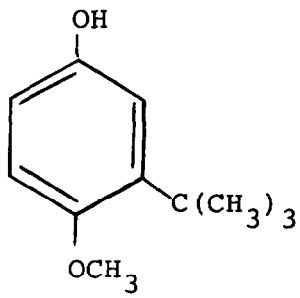
**Clinical data for BHA in cosmetic formulations indicated that they were generally nonsensitizing, nonphotosensitizing, and only minimally or mildly irritating. It is concluded that BHA is safe as a cosmetic ingredient in the present practices of use.**

## CHEMICAL AND PHYSICAL PROPERTIES

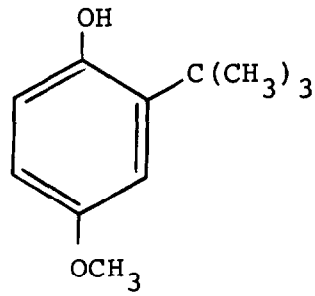
### Definition and Structure

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

* Additional information on BHA may be found in other literature reviews.[3,7,10-16]




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).[1-3]
The commercial material by definition contains not less than 98.5 percent of the empirical formula $C_{11}H_{16}O_2$.[2,4]

## 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.[5,6] 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.[2] 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.[3,7] A melting point range of 48 to 63°C has also been reported.[6] BHA is thermostable up to temperatures of 200°C.[8,9]

BHA exhibits antioxidant properties and acts synergistically with acids, butylated hydroxytoluene (BHT), propyl gallate, hydroquinone, methionine, lecithin, and thiodipropionic acid in protecting lipids against autooxidation.[17,18] 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.[19]

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.[20]

The IR spectrum of BHA has been published by Estrin.[21]

## 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-tert-butyl-2,2' dihydroxy-5,5'-dimethoxydiphenyl (A) and 2',3-di-tert-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.[22] Kurechi and Senda [23] 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.[24]

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.[25]

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-t-butyl-4-methoxy-6-nitrobenzene as the major product.[26]

BHA is reported to prevent the oxidation of such materials as vitamin $D_3$,[27] lanolin,[28] vitamin A,[29] vitamin A esters,[30] methyl oleate,[31] cod-liver oil,[32] and various fatty bases.[33] BHA reduces rancidity and improves stability by providing reactive hydrogen atoms to lipid-free radicals.[34,35] The resulting antioxidant free radical usually has several stable resonance forms, which in turn prevent further stimulation of free radical formation (Fig. 1).[36] Thus, BHA prevents lipid peroxidation by acting as a chain-breaker in autooxidation processes.[37] Theories relating to antioxidant properties of BHA in cosmetic formulations and raw materials have been reviewed by Marcinkiewicz.[38]

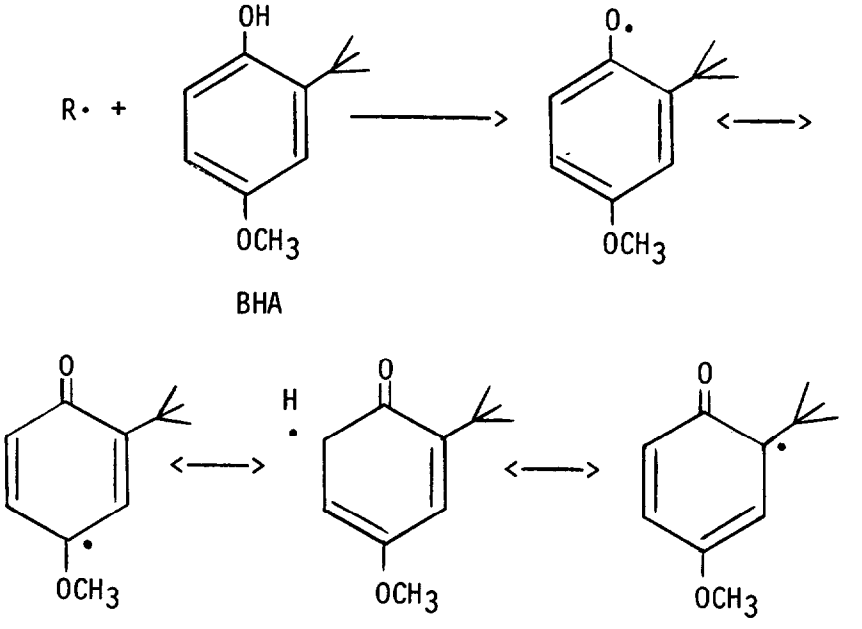



**Figure 1.** Stable resonance forms of BHA.[36]

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

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.[37]

## Analytical Methods

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

Several literature reviews relating to the analytical determination of BHA have been published.[3,76,77]

## Method of Manufacture

BHA can be synthesized either by tert-butylation of *p*-methoxyphenol[78] or by methylation of tert-butylhydroquinone.[2,78,79] BHA can also be prepared from *p*-methoxyphenol and isobutene.[17] The product is purified by distillation and subsequently supplied to cosmetic formulators in the form of tablets or flakes.[2] Lam et al.[78,80] 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[2,4]:

| | |
|---|---|
| 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.[3]

# USE

## Purpose in Cosmetics

BHA is used in cosmetic formulations as a chemical preservative[81] and as an antioxidant.[2,82]

## 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 ≤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).[83]

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.[6,11,17,19,34,35,84-86] 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.[83]

| 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 |
| *BHA* | | | | | | | | |
| 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 |
| Eyeliner | 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 |
| *BHA con't* | | | | | | | | |
| 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).

| *Food* | *Max. Level of BHA Permitted* |
|---|---|
| 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[16] 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.[11]

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.[87]

## BIOLOGICAL PROPERTIES

### Antimicrobial Properties

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

The minimum BHA concentration required to inhibit the growth of various microorganisms in liquid culture medium (pH 7.0) has been reported by Kabara[90] (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.*[36,91-101]

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).[90]

| *Microorganism* | *BHA* |
|---|---|
| *Escherichia coli* | 2000 μg/ml |
| *Pseudomonas aeruginosa* | > 5000 μg/ml |
| *Streptococcus mutans* | 125 μg/ml |
| *Streptococcus agalactiae* | 125 μg/ml |
| *Staphylococcus aureus* | 250 μg/ml |
| *Corynebacterium sp.* | 125 μg/ml |
| *Norcardia asteroides* | 250 μg/ml |
| *Saccharomyces cerevisiae* | 125 μg/ml |
| *Candida albicans* | 250 μg/ml |

conjunction with nitrite, sorbic acid, or parabens; however, in the presence of lipid or surfactant, the antimicrobial activity of BHA is greatly reduced.[95] Beggs et al.[102] 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 μg/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.*[103] 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.[93]

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.[104] The inhibitory effect of BHA on *Tetrahymena* might be caused by binding of the phenolic compound to protein or nucleoprotein.[18]

BHA at levels of 0.005 to 0.02 g per plate of solid medium[105] and 100 ppm[106] inhibited the growth of *Aspergillus flavus,* whereas 250 ppm was fungicidal to *Aspergillus parasiticus.*[99] Ahmad[107] 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.*[108]

BHA (100 μM) was also reported to be effective in inhibiting the growth of bacteriophage $\phi$ 6.[109]

Davidson[36] and Branen et al.[110] 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[111] found that BHA concentrations of $5 \times 10^{-3}M$ were toxic to cultured guinea pig melanocytes, whereas BHA at $5 \times 10^{-6}M$ 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.[112] BHA at concentrations of $10^{-2}M$ 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.[113]

## 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.[114]

Stokes and Scudder[115] 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,[116] 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[117] 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[118] 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.[119] reported that BHA stimulated prostaglandin biosynthesis (as indicated by cyclooxygenase activity) at concentrations of 30 μM but inhibited biosynthesis at 160 μM.

Volkova[120] 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 $10^{-6}$M BHA. The antioxidant interacted directly with the polypeptide kinin to impair the latter's function.[121] Posati and Pallanch[122] 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.[123] 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).[124,125] The BHA-mediated protection of rodents against the neoplastic effects of carcinogens is considered by Benson et al.[126] 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.[127,128] With regard to the last mechanism, Awasthi et al.[129] 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 Slaga and Braken,[130] 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.[131] reported that BHA (100 μM) 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.[126] Benson et al.[126,132,133] 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.[126,129] 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.[132,134] 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.[132]

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.[135,136]

Numerous other papers documenting the anticarcinogenic activity of BHA and its isomers have been published.[78,80,137-175]

### Antimutagenic Effects

In addition to inhibition of carcinogenesis, BHA has been found to inhibit mutagenesis, both in vivo and in vitro.[176-183] 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.[126,184] Dolara et al.[185] 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.[11]

Induction of drug-metabolizing enzymes by BHA is often accompanied by liver enlargement. The Select Committee on GRAS Substances[11] 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.

| *BHA Concentration* | *Test System* | *Enzyme(s)* | *Effect* | *Other Observations* | *Ref.* |
|---|---|---|---|---|---|
| 50 and 200 μM | Isolated rat liver microsome | Benzpyrene hydroxylase<br>*p*-Nitroanisole demethylase<br>Ethylmorphine demethylase<br>Aminopyrine demethylase<br>Benzphetamine demethylase | Inhibited<br>Inhibited<br>Inhibited<br>Inhibited<br>Inhibited | BHA became bound to cytochrome P-450 but did not inhibit reduced NADPH-cytochrome c reductase activity | 186 |
| 4 μg | 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<br>BHT-oxidase<br>UDPG-dehydrogenase | Transient activation<br>Transient activation<br>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<br>BHT-oxidase | Transient activation<br>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<br>Aminopyrine demethylase | No observed induction<br>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<br>Decrease in monooxygenase caused by peroxidation of membrane lipids<br>Increase in UDP glucuronosyltransferase (*p*-nitrophenol) caused by peroxidation of membrane lipids | Inhibited<br>Inhibited<br>Inhibited | | 189 |

| | | | | | |
|---|---|---|---|---|---|
| 10 mmol/L | Microsomal cell fraction of rat liver | Monooxygenase | Inhibited | | 189 |
| | | UDP glucuronosyltransferase | Inhibited | | |
| 100 mg/kg in diet | Rat blood | Sugar cholinesterase | Inhibited | Significant decreases in ketone bodies observed | 190 |
| | | Catalase | Inhibited | | |
| 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-phophatase | 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.* |
|---|---|---|---|---|---|
| | | Aminopyrine demethylase | No effect | | |
| | | Epoxide hydratase | Activated | | |
| 0.75 percent of diet for 10 days | Rat liver microsome | 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 $b_5$ 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 | | |
| | | Benzo[a]pyrene hydroxylase | Inhibited | | |
| | | Aminopyrine demethylase | Inhibited | | |
| | | Glucose-6-phosphatase | Inhibited | | |
| 0.1 or 0.5 percent of diet for 14 days | Rat liver microsome | Epoxide hydratase | Activated | Cytochrome $b_5$ concentrations were elevated whereas cytochrome P-450 concentrations remained similar to control values | 201,202 |
| | | Ethoxycoumarin deethylase | Activated | | |
| | | Arylhydrocarbon hydroxylase | No change | | |
| 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 |

| | | | | | |
|---|---|---|---|---|---|
| | 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 | | |
| 0.75 percent of diet for 8 days | 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 |
| | 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 in spleen, testis, or brain | | |
| 15 mg/day for 10 days in diet | Microsomal fraction of mouse liver | Aniline hydroxylase<br>UDP-glucuronyltransferase<br>Glucose-6-phosphatase<br>Amino-demethylase<br>Benzo[a]pyrene hydroxylase | Activated<br>Activated<br>Activated<br>Inhibited<br>Inhibited | Decreases observed in cytochrome P-450 content, whereas increases occurred in cytochrome $b_5$ content and activities of NADPH and NADH-dependent cytochrome c reductases; increased size and protein content of liver also noted | 203 |
| | Cytosol fraction of mouse liver | Glucose-6-phosphate dehydrogenase<br>UDP-glucose dehydrogenase | Activated<br>Activated | Increased protein content | |
| 5 mg/g of chow (75 mg/kg of body weight) | Mouse liver microsome | 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.)

| *BHA Concentration* | *Test System* | *Enzyme(s)* | *Effect* | *Other Observations* | *Ref.* |
|---|---|---|---|---|---|
| 15 μM | 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 | $NAD^+$-dependent alcohol dehydrogenase | Inhibited | | 172 |
| Single IP injection of 62.5, 215, or 500 mg/kg | Lung of mice | Superoxide dismutase<br>Glutathione reductase<br>Glutathione peroxidase<br>Glucose-6-phosphatase | No change<br>No change<br>No change<br>No change | | 209 |
| 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<br>Lipase | No effect<br>No effect | | 192 |
| | Mouse intestine | Enterokinase<br>Alkaline phosphatase | No effect<br>No effect | | |
| Oral doses of 500 mg/kg/day | Monkey liver microsome | Glucose-6-phosphatase<br>Nitroanisole demethylase | Inhibited<br>Activated | Slight decreases in liver succinic dehydrogenase, blood catalase and serum phosphatase were not significant; cytochrome P-450 content was not altered | 210 |

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 enchancement of detoxification of carcinogens and their metabolites may constitute part of the biochemical mechanisms by which BHA protects against chemical carcinogenesis.[132,133]

## 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.[11,211,212] 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.,[213] 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 etheral 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

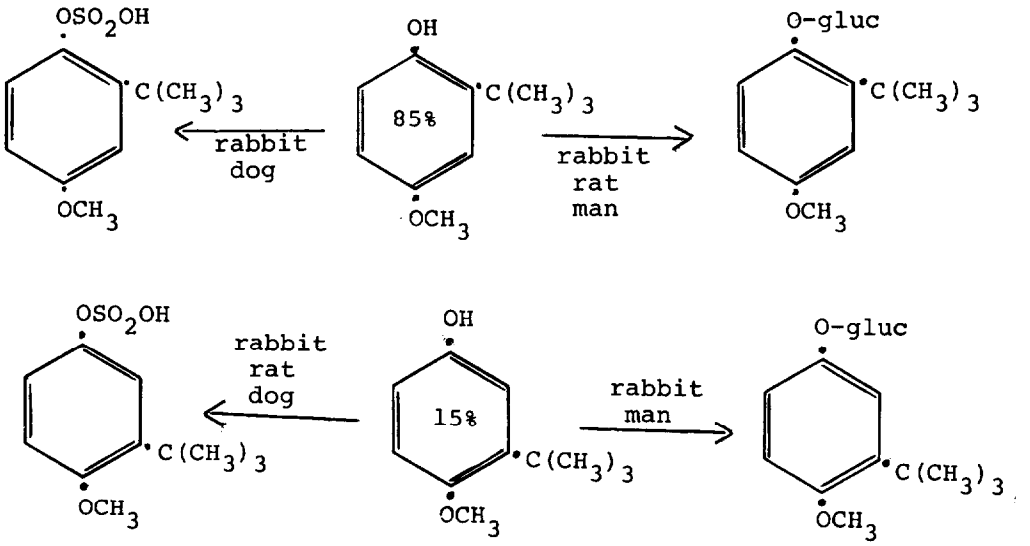



**Figure 2.** Metabolism of BHA in man and various animals.

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

| *BHA Concentration* | *Test System* | *Observations/Results* | *Reference* |
|---|---|---|---|
| $10^{-3}$ 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 endothelioid 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-$^{14}$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.)

| *BHA Concentration* | *Test System* | *Observations/Results* | *Reference* |
|---|---|---|---|
| | | 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 | |
| Single intraperitoneal injection of 62.5, 215 or 500 mg/kg | 16–24 Swiss-Webster mice/dose | 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 |
| | Guinea pig liver homogenate | BHA inhibited cellular respiration | 229 |
| 1.0 nmol/mg protein | Isolated rat liver mitochondria | BHA inhibited cation efflux during the oscillatory cycle without significant influences on either active transport or oxidative phosphorylation | 230 |
| | 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 |
| 0.8 mM or 1.0 mM | 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 |
| $10^{-4}$ M | Rat blood cell suspension | BHA caused considerable hemolysis of blood cell suspension in vitro | 234 |
| 12.5 μg/ml in culture media | *Tetrahymena pyriformis* 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 *Drosophila* 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 *Drosophila melanogaster* | 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 | *Tetrahymena pyriformis* | Protection against photodynamic toxicity of benzo[a]pyrene observed | 247 |
| $10^{-4}$ to $10^{-3}$ M or $10^{-6}$ to $10^{-5}$ M | Chinese hamster Don cells | BHA inhibited mitosis at concentrations of $10^{-4}$ to $10^{-3}$ M but not at $10^{-6}$ to $10^{-5}$ 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.)

| *BHA Concentration* | *Test System* | *Observations/Results* | *Reference* |
|---|---|---|---|
| 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 oxido-reducing 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 etheral sulfate excreted. Repeated doses of 0.5 g/2-BHA/kg were followed by a considerable increase in glucuronide and a decrease in etheral sulfate conjugation. The authors suggested that 4-0-conjugation, 0-demethylation, hydroxylation of the phenyl ring, and sidechain oxidation ($-CH_3 \rightarrow CH_2 \cdot OH$) may be involved in the metabolism of BHA. The possibility of 0-demethylation of the 2-BHA isomer as an alternative metabolic pathway was also recognized.

Investigations by Golder et al.[252] indicated that the antioxidant is rapidly excreted in urine by the albino rat. Animals given 97 μg 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[253] 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.[213] 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 4-0-glucuronide formation. In studies by Dacre et al.,[254] 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[211] 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.[255] Fasted dogs given dietary BHA doses of 50 and 250 mg/kg in the diet had ethereal sulfate and glucuronides in the urine.[256]

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 0-demethylated metabolites (about 42 percent).[174] In a second study, a single dose of 40 mg $^{14}C$-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.[257] 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 dealkylatin or hydroxylation products were detected. The time required for excretion of the administered dose varied from 23 to 50 hours.[255]

The Select Committee on GRAS Substances[11] 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.[258] 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.[259] In addition, Wilder and Kraybill[260] 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."[261]

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 $\mu$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."[262]

## 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 $LD_{50}$ for both products was $>5.0$ g/kg.[268,269] 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 $LD_{50}$ in rats was also $>5$ g/kg.[270]

**TABLE 5.** Acute Oral Toxicity.

| *Material Tested* | *Animal* | *$LD_{50}$ (g/kg)* | *Reference* |
|---|---|---|---|
| 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.[271]

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.[272]

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.[270]

## 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.[273-275]

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.[270]

### 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 × 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.[276,277]

### 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 $LD_{50}$ of the makeup formulation was determined to be $>2$ g/kg.[270]

### 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.

| *BHA Dose* | *Animal* | *Results* | *Reference* |
|---|---|---|---|
| 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 | *p*-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.)

| *BHA Dose* | *Animal* | *Results* | *Reference* |
|---|---|---|---|
| 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 $P_{450}$. After 4 weeks, activities of glucose-6-phosphatase and nitroanisole 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 $P_{450}$ levels, or hepatic protein, DNA, or RNA. Nitroanisole demethylase and glucose-6-phophatase 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.)

| *BHA Dose* | *Animal* | *Results* | *Reference* |
|---|---|---|---|
| | | animal with respect to sex cycle phases, activity of gonadotropic pituitary hormone glands, or histology of endocrine glands | |
| Mixture of propyl gallate (20 mg/kg/day) and BHA (10 mg/kg/day) for 9 months in diet | Female rats | 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 |

| | | | |
|---|---|---|---|
| | | 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 | |
| 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 | 3 beagle dogs/dose | 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 | 259 |
| Both 50 mg/kg BHA and 50 mg/kg BHT in diet for 2 years | 6 adult rhesus female monkeys | 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 | 295 |

## 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.[278]

Berry et al.[279] 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.[280]

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.[281] 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.[260] No tumors were reported in rats fed diets containing 0, 0.01, or 0.1 percent of the antioxidant for 2 years (Table 6).[282]

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

Results from one recent study suggest that BHA is carcinogenic to the forestomach of rats.[283] 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.[10]

### 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.[296] 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 μg per plate, and *S. typhimurium* strains TA-98, TA-100, TA-1535, TA-1537, and TA-1538.[297]

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).[265]

BHA at doses of 15, 150, and 1500 mg/kg in isopropyl alcohol was nonmutagenic 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.[265]

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 μg/ml produced no significant aberration in the anaphase chromosomes of human embryonic lung cultures in vitro.[265] Chromosomal aberration tests conducted on Chinese hamster fibroblasts in vitro were negative for $10^{-4}M$ BHA in ethanol.[298]

Natake et al.[299] 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.[300] 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[301] 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.[302]

Thomas et al.[303] found 0.2 mM BHA to be a "potent" enchancer of nitrous acid mutagenesis of duplex DNA in *Haemophilus influenzae*.

### Teratogenesis

Hansen and Meyer[304] 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.[304] 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.[305]

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.[306]

Clegg[307] 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 Benger 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).[308]

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.[309]

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.[82]

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.[310]

Degreff and Verhoeve[311] 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.[312] This study demonstrates a lymphocyte-mediated allergy in which patients have been sensitized after repeated local application of the chemical.[313]

Eighty-three "consecutive" patients with eczematous dermatitis were patch tested with 5 percent BHA in alcohol; all were negative for contact dermatitis.[312]

Fisherman and Cohen[314] 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.[315] reported other studies in which 37 subjects were identified as BHA- and BHT-intolerant.

Fisher[82] 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.[312]

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.[316]

### 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.[317-319] 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.[320,321]

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

* 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.

| *Type of Study* | *Material Tested* | *BHA Conc.* | *No. of Subjects* | *Procedure* | *Results* | *Reference* |
|---|---|---|---|---|---|---|
| *BHA* | | | | | | |
| 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 tc 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* |
|---|---|---|---|---|---|---|
| *BHA* (con't.) | | | | | | |
| 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 pretreatment; investigator concluded "no evidence of contact sensitization" | 328 |
| Cumulative skin irritation | Liquid makeup | 0.01% | 10 | Phillips et al.[331]: 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.[331]: 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.[331]: 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.[331]: 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 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | centrate (high irritation standard), respectively. Products were considered "essentially nonirritating" to very slightly irritating | |
| Controlled use/skin irritation | Eye makeup | 0.1% | 51 | Haynes and Estrin[334]: Product applied in eye area for four weeks under normal use conditions | 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* |
|---|---|---|---|---|---|---|
| *BHA* (con't.) | | | | | | |
| Skin irritation, sensitization, and photosensitization | Eye makeup | 0.1% | 728 | Schwartz and Peck[341] 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[343] 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.[322-324] 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.[336]

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."[325]

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.[345] 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."[326]

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.[327]

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."[328]

Four separate studies were conducted to evaluate the cumulative skin irritant properties of various cosmetic formulations containing BHA.[329,330,332,333] A modification of the procedure of Phillips et al.[331] 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.[329] 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.[330] 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);

* 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."[332] 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.[333]

Test methods described by Haynes and Estrin[334] 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.[335,336]

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.[337] 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.[338]

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.[339] 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.[340]

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.[341] 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.[342]

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.[343] 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."[342]

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.[344]

## 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 $\mu$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 $10^{-6}$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 mitochrondrial–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 $LD_{50}$ 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 $LD_{50}$s in rats of >5 g/kg, whereas an eye makeup formulation containing 0.1 percent BHA had a dermal $LD_{50}$ 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 hepatoxicity. 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.[283] 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,"[10] 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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September 9, 2003

# MEMORANDUM

To: CIR Expert Panel and Liaisons

From: Eric Hooker, M.S.
Scientific Analyst and Writer

Subject: Re-review of Butylated Hydroxyanisole (BHA)

The CIR Expert Panel issued a Final Report on the Safety Assessment of Butylated Hydroxyanisole in 1983, with the conclusion that Butylated Hydroxyanisole is safe as a cosmetic ingredient in the present practices of use.

A search was performed to identify any new safety data that may have come available since the 1983 conclusion was made. The new information is summarized in the attached document. The frequency of use data provided by the FDA has been updated. The updated concentration of use data from CTFA should be available at the September Panel Meeting. The original safety assessment is also attached for your review.

There have been a number of review articles about Butylated Hydroxyanisole in the published literature. The one that seemed most relevant to CIR's purposes (Williams et al., 1999) is attached in this package. The other review articles are listed at the end of the re-review document. All references will be brought to the September Panel Meeting.

The Panel should decide whether the new information available on Butylated Hydroxyanisole supports the original conclusion or suggests the need for a reconsideration.

# RE-REVIEW OF BUTYLATED HYDROXYANISOLE

## INTRODUCTION

Butylated Hydroxyanisole was reviewed by the CIR Expert Panel in 1983 and determined to be safe as a cosmetic ingredient in the practices of use at that time (Elder, 1984). A search of the published literature was performed to identify any new information regarding the safety of Butylated Hydroxyanisole that has come available since the Panel's 1983 conclusion.

## NOMENCLATURE

The name of Butylated Hydroxyanisole as listed in the International Cosmetic Ingredient Dictionary and Handbook, 9$^{th}$ edition, has been change to BHA (Pepe et al., 2002).

## COSMETIC USE

Table 1 lists the current historic frequency of use and historic concentration of use of BHA in cosmetic products as reported to the Food and Drug Administration (FDA). The historic concentrations of use listed are those concentrations that were actually reported. For almost all product categories listed, there were products for which the concentrations of BHA were not reported in 1981. The highest concentration of use of BHA reported to the FDA in 1981 was up to 25 % in lipstick (Elder, 1984).

Table 1. Historic and Current Frequency of Use and Historic Concentration of Use of BHA.

| Product Category (total number of products in category in 2002) | 2002 Frequency of Use (FDA, 2002) | 1981 Frequency of Use (Elder, 1984) | 1981 Reported Concentration of Use (Elder, 2002) |
|---|---|---|---|
| Baby lotions, oils, powders, and creams (60) | 1 | 1 | >0.1 - 1 % |
| Bath oils, tablets, and salts (143) | 4 | 20 | ≤0.1 % |
| Bubble baths (215) | - | 7 | ≤0.1 % |
| Other bath preparations (196) | 3 | 10 | ≤ 1 % |
| Eyebrow pencil (102) | 51 | 33 | ≤ 1 % |
| Eyeliner (548) | 399 | 75 | ≤ 1 % |
| Eye shadow (576) | 38 | 410 | ≤ 5 % |
| Eye lotion (25) | 2 | 2 | ≤0.1 % |
| Eye makeup remover (100) | 6 | 11 | ≤0.1 % |
| Mascara (195) | 18 | 65 | ≤ 1 % |
| Other eye makeup preps. (152) | 10 | 39 | ≤ 1 % |
| Colognes and toilet waters (684) | 18 | 97 | ≤ 1 % |
| Perfumes (235) | 6 | 62 | ≤ 1 % |
| Fragrance powders (273) | 2 | 12 | ≤0.1 % |
| Sachets (28) | - | 21 | ≤0.1 % |
| Other fragrance preps (173) | 10 | 24 | ≤ 1 % |
| Hair conditioners (651) | 5 | 8 | ≤0.1 % |
| Hair sprays (aerosol) (275) | - | 1 | - |
| Hair shampoos (non-coloring) (884) | - | 6 | ≤0.1 % |
| Tonics, dressings and other hair grooming aids (598) | 8 | 10 | ≤ 1 % |
| Wave sets (53) | - | 1 | - |
| Other hair coloring preps. (55) | 1 | 5 | ≤0.1 % |
| Blushers (245) | 26 | 176 | ≤ 5 % |
| Face Powders (305) | 11 | 98 | ≤ 1 % |
| Makeup foundations (324) | 30 | 119 | ≤0.1 % |
| Lipstick (962) | 279 | 1256 | ≤ **25** % |
| Makeup bases (141) | 4 | 64 | ≤ 1 % |
| Rouges (28) | 1 | 48 | ≤ 1 % |
| Makeup fixatives (20) | - | 10 | ≤0.1 % |
| Other makeup preps. (not eye) (201) | 23 | 106 | ≤ 5 % |

| Product Category | 2002 freq. | 1981 freq | 1981 conc. |
|---|---|---|---|
| Nail basecoats and undercoats (44) | 3 | 1 | ≤0.1 % |
| Cuticle softeners (19) | 2 | 2 | ≤0.1 % |
| Nail creams and lotions (15) | 1 | 4 | ≤0.1 % |
| Nail polish and enamel remover (36) | - | 1 | ≤0.1 % |
| Other manicuring preps. (55) | 4 | 2 | ≤0.1 % |
| Bath soaps and detergents (421) | 5 | 2 | ≤0.1 % |
| Deodorants (underarm) (247) | 1 | 1 | ≤0 1 % |
| Other personal cleanliness products (308) | 4 | 2 | ≤ 1 % |
| Aftershave lotions (231) | 2 | 11 | ≤ 1 % |
| Preshave lotions (14) | - | 3 | - |
| Shaving cream (aerosol) brushless and lather (134) | 10 | 8 | ≤0.1 % |
| Shaving soap (2) | - | 1 | > 0 1 - 1 % |
| Other shaving prep. products (63) | - | 3 | ≤ 1 % |
| Skin cleansing preps. (775) | 23 | 51 | ≤ 1 % |
| Face, body, and hand skin care preps (excl shaving preps ) (category no longer used) | - | 77 | ≤ 1 % |
| Face and neck skin care preps. (310) | 15 | - | - |
| Body and hand skin care preps. (840) | 72 | - | - |
| Hormone skin care preps (category no longer used) | - | 1 | - |
| Foot powders and sprays (35) | 1 | - | - |
| Moisturizing skin care preps (905) | 51 | 111 | ≤ 1 % |
| Night skin care preps (200) | 26 | 30 | ≤ 1 % |
| Paste masks (mud packs) (271) | 3 | 6 | ≤ 1 % |
| Skin lighteners (category no longer used) | - | 11 | ≤0.1 % |
| Skin fresheners (184) | 2 | 6 | ≤0.1 % |
| Wrinkle smoothers (category no longer used) | - | 6 | ≤0 1 % |
| Other skin care preps (725) | 30 | 42 | ≤ 1 % |
| Suntan gels, creams, and liquids (131) | 7 | 27 | ≤ 1 % |
| Indoor tanning preps. (71) | 1 | 2 | ≤0 1 % |
| Other suntan preps. (38) | 5 | 9 | ≤0.1 % |
| Total Number of Cosmetic Products Containing BHA | 1244 | 3217 | |

## ABSORPTION AND METABOLISM

Schumann, R. In vitro absorption of butylated hydroxyanisole through human skin. *J. Soc. Cosmet. Chem.* 42: 335-340.

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

Castelli, M. G., E. Benfenati, R. Pastorelli, M. Salmona, and R. Fanelli. 1984. Kinetic of 3-tert-butyl-4-nydroxyanisole (BHA) in man. *Food Chem. Toxicol.* 22(11): 901-904.

Four human volunteers were given a single oral dose of 5 or 30 mg BHA in olive oil. Plasma concentration of BHA peaked at 73.03 ng/ml 108.75 minutes after the 30 mg dose and at about 14.14 ng/ml 142 minutes after the 5 mg dose. The half-life of BHA in plasma was 2.79 hours fot the 5 mg dose and 2.96 hours for the 30 mg dose. About 20 % of the administered dose was excreted as BHA-glucuronide in the urine within 24 hours. Only 0.03 % of the administered dose was excreted as free BHA.

Hirose, M., A. Hagiwara, K. Inoue, T. Sakata, N. Ito, H. Kaneoko, A. Yoshitake, and J. Miyamoto. 1987. Metabolism of 2- and 3-tert-butyl-4-hydroxyanisole (2- and 3-BHA) in the rat (I): excretion of BHA in urine, feces, and expired air and distribution of BHA in the main organs. *Toxicology*. 43: 139-147.

Within 2 days after a single oral dose of labelled BHA at 1 g/kg body wt, 87-96% of the $^{14}C$ was excreted, mainly in the urine with smaller amounts in the feces and expired air. More $^{14}C$ was found in the tissues of rats given the methoxy-labelled compounds. The distributions of $^{14}C$ in the forestomach and the glandular stomach were similar. At 168 h after treatment, more $^{14}C$ was found in the forestomach of rats given 2-BHA than in that of rats given 3-BHA. These results indicate that excretion of BHA is rapid, that 4-O-methyl demethylation may take place readily and that

demethylated methyl group may become distributed non-specifically in tissues. The carcinogenic or toxic action of BHA on the forestomach does not seem to be due accumulation of BHA in the forestomach.

## TOXICOLOGY

### ACUTE TOXICITY

Della-Corte, L and G. Sgaragli. 1984. 2-t-Butyl -4-methoxyphenol (BHA) acute toxicity in rodents: Influence of the administration route. *Pharmcaological Research Communications*. 16(10): 1041-1047.

The $LD_{50}$ values of BHA were reported as follows:

Mice, oral: 1,670 mg/kg BHA in DMSO and 1583 mg/kg BHA in olive oil.

Mice, i.p.: 29 mg/kg BHA in DMSO and 622 mg/kg BHA in olive oil.

Rats, oral: 2910 mg/kg in DMSO and 2960 mg/kg BHA in olive oil.

Rats, i.p.: 32 mg/kg BHA in DMSO and 621 mg/kg BHA in olive oil.

Iverson, F., J. Truelove, E. Nera, E. Lok, D. B. Clayson, and J. Wong. 1986. A 12-week study of BHA in the cynomolgus monkey. *Food Chem. Toxicol.* 24(10/11): 1197-1200.

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

Romero, F. J., J. Romá, F. Bosch-Morell, Belén Romero, J. Segura-Aguilar, A. Lombart-Bosch, and L. Ernster. 2000. Reduction of brain antioxidant defense upon treatment with butylated hydroxyanisole (BHA) and Sudan III in Syrian golden hamster. *Neurochemical Research* 25(3): 389-393.

BHA, at 0.7 % mixed in the feed of Syrian golden hamsters for two weeks, induced superoxide dismutase in the liver but not in the brain. BHA reduced DT-

diaphorase activity in the brain by 40 %. Glutathione (GSH)-related enzyme activities in the brain were not affected by BHA, but BHA increased GSH S-transferase and GSH reductase in the liver. The permeability of the blood brain barrier to BHA is limited.

David, M., G. Horvath, I. Schimke, M. M. Mueller, and I. Nagy. 1993. Effects of the antioxidant butylated hydroxyanisole on cytosolic free calcium concentration. *Toxicology* 77: 115-121

Twenty seconds of *in vitro* exposure to 1 mM BHA caused increases in intracellular free calcium in human umbilical endothelial cells, rat cardiomyocytes, rat pituitary cells, baby hamster kidney cells, and human granulocytes. The absence (0.0 mM) or presence (2.2 mM) of extracellular calcium did not affect the BHA-induced changes in intracellular free calcium, meaning BHA causes the release of calcium from intracellular stores. After the removal of 1 mM BHA, intracellular free calcium remained elevated in the pituitary cells and cardiomyocytes when extracellular calcium was 2.2 mM. Intracellular free calcium declined after removal of the BHA in all other cell types regardless of extracellular calcium and in pituitary cells and cardiomyocytes with no extracellular calcium. These data suggest that BHA opens some calcium channel(s) in the cell membrane pituitary cells and cardiomyocytes, but not in the other cell types tested.

Jayalakshmi, C. P. and J. D. Sharma. 1986. Effect of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on rat erythrocytes. *Environmental Research*. 41: 235-238.

Erythrocytes from healthy adult rats were exposed to 0.02 to 1.00 % BHA *in vitro* for 1 to 24 minutes, and the percentage of hemolysis was recorded. BHA caused dose- and time-dependent increases in the percent hemolysis in erythrocytes. The peak percent hemolysis occurred with 0.75 % BHA exposure for 20 and 24 minutes.

## REPRODUCTIVE TOXICOLOGY

Würtzen G and P Olsen. 1986. BHA study in pigs. *Food Chem. Toxicol* 24(10/11): 1229-1233.

BHA was given to pregnant SPF pigs in doses of 0, 50, 200 and 400 mg/kg body weight/day from mating to day 110 of the gestation period. The BHA was mixed in the diet. Caesarean section was performed on gestation day 110. BHA affected neither the reproduction data nor the incidence of defects in the foetuses. Significantly lower weight gain was observed in the group of dams on the highest dose. Absolute and relative organ weights for the liver and thyroid gland showed a dose-related increase. Proliferative and parakeratotic proliferative changes of the stratified epithelium of the stomach were found in both control and treated pigs. In addition, proliferative and parakeratotic changes of the esophageal epithelium were observed in a few pigs in the two groups on the highest doses. Papillomas were not found, and no changes of the glandular part of the stomach were observed.

## CARCINOGENICITY

Witschi, H. R and D. G. Doherty. 1984. Butylated hydroxyanisole and lung tumor development in A/J mice. *Fundam. Appl. Toxicol.* 4: 795-801.

A diet containing 0.75% butylated hydroxyanisole (BHA) did not enhance the development of lung tumors in A/J mice if fed for 8 weeks after administration of urethan, benzo[a]pyrene (B[a]P), or dimethylnitrosamine (DMN). Prefeeding animals with BHA partially protected animals against the tumorigenic effect of urethan and B[a]P. Partial protection was also seen in animals given B[a]P and then exposed to BHA in the diet. The two isomers of BHA (3-tert.-butyl-4-hydroxyanisole and 2-tert.-butyl-4-hydroxyanisole) were synthesized and injected i.p. They failed to enhance lung tumor development. It was concluded that BHA is not a promoting agent for lung tumors in mice.

Hirose, M., A. Hagiwara, T. Masui, K. Inoue, and N. Ito. 1986. Combined effects of butylated hydroxyanisole and other antioxidants in induction of forestomach lesions in rats. *Cancer Letters*. 30: 169-174.

BHA at 1 % in diet for one week induced hyperplasia of the forestomach of rats. Co-treatment with the antioxidants α-tocopherol, ellagic acid, propyl gallate, ethoxyquin, sodium L-ascorbate, or 3,3'-thiodopropionic acid increased the induction of hyperplasia. The authors suggested that the induction of hyperplasia in rats by BHA may not be related to a free radical reaction.

Hirose, M. A. Masuda, Y. Kurata, E. Ikawa, Y. Mera, and N. Ito. 1986 Histological and autoradiographic studies on the forestomach of hamsters treated with 2-tert-butylated hydroxyanisole, 3-tert-butylated hydroxyanisole, crude butylated hydroxyanisole, or butylated hydroxytoluene. *J. Natl. Cancer Inst*. 76(1): 143-150.

The inductions of hyperplasia and neoplastic lesions in the forestomach of Syrian golden hamsters by 1.0 % 2-tert-butylated hydroxyanisole (2-tert-BHA), 1.0 % 3-tert-butylated hydroxyanisole(3-tert-BHA), and 1.0 % crude BHA were compared histopathologically and autoradiographically. In hamsters fed the 2-tert-BHA diet, severe hyperplasia developed from week 4, reaching a maximum level in week 16 of 0.56 cm/10 cm basement membrane (bm), and papillomatous lesions appeared in week 16 (0.13 cm/10 cm bm). In hamsters fed 3-tert-BHA or crude BHA, severe hyperplasia developed from week 1, which reached a maximum level in week 4 of 3.63 cm/10 cm bm with 3-tert-BHA and 5.10 cm/10 with crude BHA; it then decreased. Papillomatous lesions were found in week 3 in hamsters fed 3-tert-BHA and in week 4 in hamsters fed crude BHA; they increased to maximum levels in week 16 of 0.50 cm/10 cm bm with 3-tert-BHA and 0.29 cm/10 cm bm with crude BHA. The authors suggested that the tumorigenic action of crude BHA on hamster forestomach is largely due to 3-tert-BHA.

Masui, T., M. Hirose, K. Imaida, S. Fukushima, S. Tamano, and N. Ito. 1986. Sequential changes of the forestomach of F344 rats, Syrian golden hamsters, and B6C3F1 mice treated with butylated hydroxyanisole *Jpn. J. Cancer Res.* 77: 1083-1090.

BHA was mixed in rodent diet and fed to F344 rats, Syrian golden hamsters, and $B6C3F_1$ mice eight to 104 weeks. Rats and hamsters were given feed containing 0, 1.0, or 2.0 % BHA, and mice were given feed containing 0, 0.5, or 1.0 % BHA (n = 150 animals/species/dose group). BHA caused dose-dependent increases in the incidence of forestomach hyperplasia, papilloma, and squamous cell carcinomas. Hamsters were most sensitive followed by rats, and mice were the least sensitive.

Williams, G. M., C. X. Wang, and M. J. Iatropoulos. 1990. Toxicity studies of butylated hydroxyanisole and butylated hydroxytoluene. II chronic feeding studies. *Food Chem. Toxicol.* 28(12): 799-806.

F344 rats fed diet containing 12,000 ppm BHA for 110 weeks developed papillomas and mild to moderate hyperplasia of the squamous stomach and mild to moderate dysplasia of the glandular stomach.

## GENETIC TOXICITY

Hageman, G. J., H. Verhagen, and J. C. S. Kleinjans. 1988. Butylated hydroxyanisole, butylated hydroxytoluene and tert-butylhydroquinone are not mutagenic in the Salmonella/microsome assay using new tester srains. *Mutat. Res.* 208: 207-211.

BHA (1 to 1000 µg/plate) was not mutagenic in Salmonella strains TA97, TA100, TA102, or TA104 with or without S9 microsomal activation. However, cytotoxic effects were seen at 500 and 1000 µg/plate.

Matsuoka, A., M. Matsui, N. Miyata, T. Sofuni, and M. Ishidate, Jr. 1990. Mutagenicity of 3-tert-butyl-4-hydroxyanisole (BHA) and its metabolites in short-term tests in vitro. *Mutation Research.* 241: 125-132.

BHA (0.5 to 250 µg/plate) was not mutagenic in Salmonella strains TA100, TA98, TA102, or TA97 with or without S9 microsomal activation. However, lethal cytotoxic effects were seen at 100 µg/plate without S9 and at 250 µg/plate with S9 mix. BHA (.125 mg/ml) in the presence of S9 mix induced chromosomal aberrations in Chinese hamster fibroblast cells.

Williams, G. M., C. A. McQueen, and C. Tong 1990 Toxicity studies of butylated hydroxyanisole and butylated hydroxytoluene I Genetic and cellular effects *Food Chem Toxicol.* 28(12): 793-798.

BHA was negative for genotoxicity the hepatocyte primary culture/DNA repair test (0.01 to 1.0 µg/ml, toxic at 5 µg/ml); Salmonella/microsome mutagenesis test (1 to 100 µg/plate); adult rat liver cell/ HGPRT mutagenicity test (60 to 90 µg/ml); and the Chinese hamster ovary/sister chromatid exchange test (5 to 50 µg/ml, toxic at 500 µg/ml).

Kanazawa, K. and M. Mizuno. 1992. Butylated hydroxyanisole produces both mutagenic and desmutagenic derivatives under gastric conditions. *Int. J. Tissue Reac.* 14(5): 211-218.

BHA was treated with simulated gastric conditions, and the metabolites were tested for mutagenicity and anti-mutagenic potential in a Salmonella mutagenicity assay. The BHA metabolites 2-tert-butyl-p-quinone and 3,3'-di-tert-butyl-biphenyldiquinone-(2,5,2',5') were each base-substitution mutagens. 2,6-Di-tert-butyl-8-hydroxy-dibenzofuran-1,4-quinone protected Salmonella strains from the mutagenic effects of Trp-P-2. Thus, BHA can produce both mutagenic and anti-mutagenic metabolites under gastric conditions.

Schilderman, P. A. E. L., E. Rhijnsburger, I. Zwingmann, and J. C. S. Kleinjans. 1995. Induction of oxidative DNA damage and enhancement of cell proliferation in human lymphocytes in vitro by butylated

hydroxyanisole. *Carcinogenesis*. 16(3): 507-512.

BHA, at 50 µM, induced a dose dependent increase in cell proliferation of phytohaemagglutinin-stimulated lymphocytes, however 100 µM BHA did not induce oxidative DNA damage. tert-Butylquinone, at 100 µM, and 50 µM tert-butylhydroquinone, metabolites of BHA, increased formation of 7-hydroxy-8-oxo-2'-deoxyguanosine in lymphocytes.

Sakai, A., N. Miyata, and A. Takahashi. 1997. Promoting activity of 3-tert-4-hydroxyanisole (BHA) in BALB/3T3 cell transformation. *Cancer Letters*. 115: 213-220.

BHA (5 to 20 µg/ml) dose dependently enhanced the cell transformation activity of 3-methylcholanthrene in BALB/3T3 mouse embryo cells. However, BHA did not increase transformations without the presence of another initiator or promotor. BHA did not increase cell proliferation.

## ANTI-CARCINOGENIC ACTIVITY

Richer, N., M. Marion, and F. Denizeau. 1989. Inhibition of binding of 2-acetylaminofluorene to DNA by butylatedhydroxytoluene and butylated hydroxyanisole in vitro. *Cancer Letters*. 47: 211-216.

BHA inhibited the binding of 2-acetylaminofluorene (2-AAF) to DNA in calf thymus and rat hepatocyte cultures.

Yeh, C., J. Chung, H. Wu, Y. Li, Y. Lee, and C. Hung. 2000. Effects of butylated hydroxyanisole and butylated hydroxytoluene on DNA adduct formation and arylamines N-acetyltransferase activity in PC-3 cells (numan prostate tumor) in vitro. *Food Chem. Toxicol*. 38: 977-983.

BHA, at 0.8, 8, 80, and 160 µM, dose dependently inhibited N-acetyltransferase activity in PC-3 human prostate tumor cells. BHA, at 8 mM, inhibited the formation of 2-aminofluorene-DNA adducts in human prostate tumor cells.

Chang, S., G. Chen, C. Yeh, C. Hung, S. Lin, and J. Chung. 2001. Effects of butylated hydroxyanisole and

butylated hydroxytoluene on the DNA adduct formation and arylamines N-acetyltransferase activity in human colon tumor cells. *Anticancer Research*. 21: 1087-1094.

BHA, at 8, 80, and 160 µM, was a noncompetitive inhibitor of N-acetyltransferase activity in Colo 205 human colon tumor cells. BHA, at 80 µM, inhibited the formation of 2-aminofluorene-DNA adducts in human colon tumor cells.

Slameňová, D., E. Horvánthová, S. Robichová, L Hrušovská, A. Gábelová, K. Kleibl, J. Jakubiková, and J. Sedlák. 2003. Molecular and cellular influences of butylated hydroxyanisole on Chinese hamster V79 cells treated with N-methyl-N'-nitro-N-nitrosoguanidine: Antimutagenicity of butylated hydroxyanisole. *Environ. Mol. Mutagen*. 41: 28-36.

The antioxidant activity of 0.25 or 0.5 mM BHA protected Chinese hamster V79 cells from the mutagen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The higher concentration of BHA was cytotoxic. BHA did not prevent MNNG-induced DNA strand breaks, but it did prevent their rejoining.

Ishii, T., K. Itoh, J. Akasaka, T. Yanagawa, S. Takahashi, H. Yoshida, S. Bannai, and M. Yamamoto. 2000. Induction of murine intestinal and hepatic peroxiredoxin MSP23 by dietary butylated hydroxyanisole. *Carcinogenesis*. 21(5): 1013-1016.

BHA fed to mice at 0.7 % BHA for seven days induced the activity of and mRNA for peroxiredoxin I (MSP23) in the liver and small intestine. The MSP23 enzyme may be important to protect cells and tissues against toxic electrophiles and reactive oxygenated species.

## HEPATIC EFFECTS

Hazelton, G. A., J. J. Hjelle, and C. D. Klaassen 1986 Effects of butylated hydroxyanisole on acetaminophen hepatotoxicity and glucuronidation in vivo. *Toxicol. Appl Pharmacol*. 83: 474-485.

BHA (600 to 800 mg/kg/day) in diet prevented hepatotoxicity induced by 600 mg/kg i.p. acetaminophen. BHA increased the rate of acetaminophen elimination from the blood by increasing the glucuronidation of acetaminophen. Hepatic UDP-

glucuronosyltransferase activity and hepatic UDP-glucuronide concentrations were increased by BHA.

Buetler, T. M., E. P. Gallagher, C. Wang, D. Stahl, J. D. Hayes, and D. L. Eaton. 1995. Induction of phase I and Phase II drug-metabolizing enzyme mRNA, protein, and activity by BHA, ethoxyquin, and oltipraz. *Toxicol. Appl. Pharmacol.* 135. 45-57.

BHA, at 0.75 % in the diet of male Sprague-Dawley rats for three days, induced the hepatic enzymes CYP 1B, glutathione S-transferase, γ-glutamycysteine synthetase, and quinone oxidoreductase, but did not induce CYP 1A.

Sun, B. and M. Fukuhara. 1997. Effects of co-administration of butylated hydroxytoluene, butylated hydroxyanisole and flavinoids on the activation of mutagens and drug-metabolizing enzymes in mice. *Toxicology.* 122: 61-72.

Co-administration of 0.2 % BHA and 0.1 % flavone or 0.1 flavanone resulted in additive effects on the activation of aflatoxin $B_1$ and benzo[a]pyrene, but theer was no additive effect in the activation of N-nitrosodimethylamine. Co-administration of 0.2 % BHA and 0.1 % flavanone elevated the levels of proteins and enzyme activities associated with CYP1A.

## CLINICAL

Orton, D. L. and S. Shaw. 2001. Allergic contact dermatitis from pharmaceutical grade CHA in Timodine®, with no patch test reaction to analytical grade BHA. *Contact Dermatitis.* 44:191-192.

Two patients had contact dermatitis reactions to pharmaceutical grade BHA (2 %) in Timodine® cream. However, the two patients had negative reactions to analytical grade BHA (2 %) in patch tests. The authors could not explain the discrepancy.

White, I. R., C. R. Lovell, and E. Cronin. 1984. Antioxidants in cosmetics. *Contact Dermatitis.* 11: 265-267.

Seven patients who had allergic contact dermatitis to some cosmetics and

toiletries were found to be sensitive to 1 % BHA.

Tosti, A., F. Bardazzi, F. Valeri, and R. Russo 1987. *Contact dermatitis from butylated hydroxyanisole. Contact Dermatitis*. 17(4): 257-258.

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

## OTHER ASSESSMENTS

International Agency for Research on Cancer (IARC) 1986. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation. Vol 40. Lyon France:IARC.

IARC reviewed the carcinogenic potential of BHA. While there were no human data available, there was sufficient evidence to demonstrate carcinogenicity in animals. In its overall evaluation, the IARC determined that BHA is possibly carcinogenic in humans.

National Toxicology Program. 2002. Report on Carcinogens, Tenth Edition. U.S. Department of Health and Human Services, Public Health Service. (5 pages).

NTP determined that BHA is "reasonably anticipated to be a human carcinogen." The NTP report cited studies in which BHA in the diet induced papillomas and squamous cell carcinomas in the forestomach of rats, mice, and hamsters. The NTP found no evidence that BHA administered topically, subcutaneously, or intraperitoneally was carcinogenic to mice.

Williams, G. M., M. J. Iatropoulos, and J. Whysner. 1999. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. *Food Chem. Toxicol.* 37: 1027-1038.

BHA at food additive levels were determined to represent no cancer hazard and

may actually protect humans against cancer and provide other health benefits. The authors suggested that the carcinogenicity of BHA seen only in rodent forestomach is species-specific and not relevant to humans (humans do not have a forestomach). Furthermore, human exposure (<0.1 mg/kg/day) is far below the lowest observed effect level for forestomach hyperplasia (230 mg/kg/day) in rats. **A copy of this safety assessment is included with this review.**

Other published reviews of Butylated Hydroxyanisole include:

Anonymous. 1999. Safety evaluation of certain food additives. Evaluation of national assesments of intake of BHA. *WHO Food Additives Series*. 42: 415-428.

Poulsen. 1991. Safety evaluation of substances consumed as technical ingredients (food additives). *Food Addit. Contam*. 8(2): 125-134.

Waters et al. 1990. Antimutagenicity profiles for some model compounds. *Mutat Res*. 238(1): 57-85.

Wurtzen. 1993. Scientific evaluation of the safety factor for the acceptable daily intake (ADI). Case study: butylated hydroxyanisole. *Food Addit. Contam*. 10(3): 307-314.

Clayson et al. 1993. The impotance of cellular proliferation induced by BHA and BHT. *Toxicol. Ind. Health*. 9(1-2): 231-342.

Hocman. 1988. Chemoprevention of cancer: phenolic antioxidants (BHT, BHA). *Int. J. Biochem*. 29(7): 639-651

Ito et al. 1985. Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. *Crit. Rev. Toxicol*. 15(2): 109-150.

Whysner & Williams. 1996. Butylated hydroxyanisole mechanistic data and risk assesment: conditional species-specific cytotoxicity, enhance cell proliferation, and tumor promotion. *Pharmacology & Therapeutics*. 71(1-2): 137-151.

Conning & Phillips. 1986. Comparative metabolism of BHA, BHT and other phenolic antioxidants and its toxicological relevance. *Food Chem. Toxicol*. 24(10-11): 1145-1148.

Moch. 1986. Pathology of BHA- and BHT-induced lesions. *Food Chem. Toxicol*. 24(10-11): 1167-1169.

Grice. 1988. Safet evaluation of butylated hydroxyanisole from the perspective of effects on forestomach and oesophageal squamous epithelium. *Food Chem. Toxicol*. 26(8): 717-724.

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Food and Drug Administration (FDA). 2002. Frequency of use of cosmetic ingredients. *FDA database*. Washington:FDA.

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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 ≤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) % |
|---|---|---|---|---|
| **Baby care** | | | | |
| Lotions, oils, powders, and creams | 1 | 1 | >0.1–1 | 0.0001 |
| **Bath** | | | | |
| 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 |
| **Eye makeup** | | | | |
| 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 |
| **Fragrances** | | | | |
| 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 |
| **Noncoloring hair care** | | | | |
| 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 |
| **Hair coloring** | | | | |
| Other hair coloring | 5 | 1 | ≤0.1 | — |
| **Makeup** | | | | |
| 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 |
| **Nail care** | | | | |
| 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) % |
|---|---|---|---|---|
| **Oral hygiene** | | | | |
| Dentifrices | — | — | — | 0.01 |
| **Personal hygiene** | | | | |
| Underarm deodorants | 1 | 1 | ≤0.1 | 0.002 |
| Other personal hygiene | 2 | 4 | ≤1 | 0.002 |
| **Shaving** | | | | |
| 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 |
| **Skin care** | | | | |
| 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 |
| **Suntan** | | | | |
| Suntan gels, creams, and liquids | 27 | 7 | ≤1 | 0.1 |
| Indoor tanning | 2 | 1 | ≤0.1 | — |
| Other suntan | 9 | 5 | ≤0.1 | — |
| **Total uses/ranges for BHA** | **3217** | **1224** | **≤0.1–25** | **0.000004-0.2** |

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

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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~~

**Concentration of Use by FDA Product Category – BHA**

| Product Category | Maximum Concentration of Use |
|---|---|
| Eyebrow pencils | 0.05% |
| Eyeliners | 0.05% |
| Eye shadows | 0.000086-0.05% |
| Mascaras | 0.03% |
| Other fragrance preparations | 0.001% |
| Hair conditioners | 0.0084% |
| Hair sprays<br>Aerosol | <br>0.00000004% |
| Shampoos (noncoloring) | 0.0024% |
| Face powders | 0.05% |
| Foundations | 0.02% |
| Lipstick | 0.05% |
| Other manicuring preparations | 0.15% |
| Dentifrices | 0.00045% |
| Bath soaps and detergents | 0.0006-0.0022% |
| Deodorants<br>Not spray<br>Aerosol | <br>0.00076%<br>0.000051% |
| Skin cleansing (cold creams, cleansing lotions, liquids and pads) | 0.00025% |
| Face and neck products<br>Not spray | <br>0.00013-0.013% |
| Body and hand products<br>Not spray | <br>0.0021% |
| Night products<br>Not spray | <br>0.00001% |

Information collected in 2022-2023

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