EVALUATION OF THE HEALTH ASPECTS OF BUTYLATED HYDROXYANISOLE AS A FOOD INGREDIENT

1978

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D. C.

Contract No. FDA 223-75-2004
EVALUATION OF THE HEALTH ASPECTS OF BUTYLATED HYDROXYANISOLE AS A FOOD INGREDIENT

1978

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004

Life Sciences Research Office
Federation of American Societies for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Background information</td>
<td>3</td>
</tr>
<tr>
<td>III. Consumer exposure data</td>
<td>4</td>
</tr>
<tr>
<td>IV. Biological studies</td>
<td>8</td>
</tr>
<tr>
<td>V. Opinion</td>
<td>23</td>
</tr>
<tr>
<td>VI. References cited</td>
<td>25</td>
</tr>
<tr>
<td>VII. Scientists contributing to this report</td>
<td>34</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

This report concerns the health aspects of using butylated hydroxyanisole as a food ingredient. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1973.* To assure completeness and currency as of the date of this report, this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; recent literature searches by the Toxicology Information Response Center, Oak Ridge, Tennessee; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, announcement was made in the Federal Register of June 7, 1977 (42 FR 29105-29107) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using butylated hydroxyanisole as a food ingredient. Three requests were received, but one request was withdrawn. Those who requested opportunity to present data, information and views are identified at the end of this report. The material presented at the hearing has been considered by the Select Committee in reaching its final conclusions.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (2) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (2) recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB-223 863/2) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that its conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on butylated hydroxyanisole and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of this substance under the Federal Food, Drug, and Cosmetic Act.
Butylated hydroxyanisole (BHA) is a white or slightly yellow waxy crystalline solid with an aromatic odor. It is a synthetic product and is not known to occur in nature (1,3). The commercial material is defined as a mixture predominantly of 3-tert-butyl-4-methoxyphenol (2-tert-butyl-4-hydroxyanisole, or 2-BHA) and 15 percent or less of 2-tert-butyl-4-methoxyphenol (3-tert-butyl-4-hydroxyanisole, or 3-BHA) and contains not less than 98.5 percent of the two isomers (3). The Food Chemicals Codex (3) specifies that BHA should assay not less than 98.5 percent C₁₁H₁₆O₂ with limits on impurities for arsenic at 3 ppm and heavy metals (as lead) at 10 ppm. Residue on ignition is limited to not more than 0.01 percent.

BHA is one of several phenolic compounds used in foods because of their antioxidant properties (1,3,4,5). Most fats, oils, and fat-containing foods are susceptible to rancidification and other oxidative reactions that produce compounds with undesirable taste and odor. Lipid peroxidation is autocatalytic and proceeds as complex chain reactions that vary with the substrate, temperature, light, availability of oxygen, and the presence or absence of oxidation catalysts. Antioxidants such as BHA act as "chain breakers" in the antioxidation processes and are effective under the usual conditions of processing, storage and use of fat-containing foods (6). The BHA mixture melts between 48° and 63°C and boils above 264°C (3,6). At concentrations normally used in foods as an antioxidant, BHA is degraded when fats are heated to 150°C or above; direct loss by volatilization under these conditions is negligible (7). In deep-fat frying, BHA and other phenolic antioxidants are lost by steam distillation (6).

BHA was first used in foods in 1947 (1). By itself, or in combination with butylated hydroxytoluene (BHT), propyl gallate, or other antioxidants, BHA is classified as generally recognized as safe (GRAS) as a chemical preservative [21 CFR 182.3169](2). In GRAS usage, the content of total antioxidants may not exceed 0.02 percent of the total fat and oil content of the particular food. The meat inspection regulations of the U.S. Department of Agriculture place limits of not more than 0.01 percent of total product weight for total antioxidants including BHA in certain meat products (8). In addition to use as a GRAS substance, BHA is specifically regulated as an antioxidant for certain foods [21 CFR 172.110], chewing gum [21 CFR 172.615], and can be a component of de-foaming agents [21 CFR 173.340] (2). BHA may be added to food packaging materials [21 CFR 181.24], as well as adhesives [21 CFR 175.105] and lubricants [21 CFR 178.3570] that may come in contact with food (2). However, this report concerns only the use of BHA as a GRAS substance: that is, as an antioxidant in foods where the total antioxidant content is limited to 0.02 percent of the fat and oil content.
III. CONSUMER EXPOSURE DATA

A subcommittee of the National Research Council (NRC) surveyed
manufacturers by questionnaire concerning the addition of GRAS substances
to foods and estimated the possible daily intake of these substances within
various age groups (9). Based on information supplied by those manufac-
turers who reported adding the substance to at least one product in a food
category, a weighted mean was calculated for the usual percentage addition
of the substance to foods in the categories. Weighted means of the usual
levels of addition of BHA to various food groups are indicated in Table I.
An entry in the table does not mean that all foods in the category contain
added BHA or that any one product contains the particular level indicated.

From Market Research Corporation of America data on the mean
frequency of eating foods by food category, U.S. Department of Agriculture
data on mean portion size of foods in these categories, and the assumption
that all food products within a category contain the substance at the level
shown in Table I, the NRC subcommittee estimated possible average daily
intake to be 12.7 mg per day for individuals more than 2 years old (Table II).
Such an assumption is likely to lead to overestimates of intake. The NRC
subcommittee has recognized that in most cases its calculations of possible
intakes are overstated, often by considerable margins.* The average es-
timated total dietary intakes are likely to be much higher than would be the
intakes achieved through consumption of a diet consisting only of processed
foods to which the substances had been added at the maximum levels.

Comparison of the total amounts of BHA added to foods in 1960 with
the amounts reported for 1970 shows approximately a threefold increase
(Table III). However, it is not possible to determine from the available
data whether this represents an increase in the levels of addition or a greater
number of foods in each category to which BHA is being added. Because the
data in Table III include all food uses and do not represent use of BHA as a
GRAS food ingredient alone, it is evident that use as a GRAS food ingredient
is likely to result in less than the estimated 5.6 mg daily per capita consump-
tion. Thus, the NRC subcommittee estimate of possible average daily intake
of 12.7 mg for individuals more than 2 years old (Table II) is clearly too high.
However, assuming an average daily intake of 5.6 or 12.7 mg, an adult would
be consuming about 0.1 or 0.2 mg per kg body weight, respectively.

*An explanation for such overestimates is detailed in Section XI,
"Significance and Use of Data in Safety Evaluations," of the NRC subcom-
mittee's report (9). The Select Committee finds this explanation reasonable
and concurs in the first recommendation in Section XII of the same report,
that "In order to conduct a more accurate survey of the intake of substances
used in food processing, food consumption data collected specifically for
this purpose are needed."
TABLE I

Level of Addition of Butylated Hydroxyanisole to Foods by Food Category (9)

<table>
<thead>
<tr>
<th>Food category</th>
<th>Weighted mean (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods, baking mixes</td>
<td>15.8</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>30.6</td>
</tr>
<tr>
<td>Grain products such as pastas or rice dishes*a</td>
<td>1.0</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>137.7</td>
</tr>
<tr>
<td>Milk products*a</td>
<td>51.9</td>
</tr>
<tr>
<td>Cheese*a</td>
<td>0.1</td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td>2.0</td>
</tr>
<tr>
<td>Processed fruits, juices and drinks*a</td>
<td>6.0</td>
</tr>
<tr>
<td>Meat products</td>
<td>42.0</td>
</tr>
<tr>
<td>Processed vegetables, juices</td>
<td>1.1</td>
</tr>
<tr>
<td>Soft candy</td>
<td>20.2</td>
</tr>
<tr>
<td>Sugar, confections*a</td>
<td>17.0</td>
</tr>
<tr>
<td>Jams, jellies, sweet spreads*a</td>
<td>0.6</td>
</tr>
<tr>
<td>Sweet sauces, toppings, syrups*a</td>
<td>70.0</td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td>9.9</td>
</tr>
<tr>
<td>Soups, soup mixes</td>
<td>2.2</td>
</tr>
<tr>
<td>Snack foods</td>
<td>22.3</td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>0.7</td>
</tr>
<tr>
<td>Beverages, alcoholic</td>
<td>0.8</td>
</tr>
<tr>
<td>Nuts, nut products</td>
<td>51.0</td>
</tr>
<tr>
<td>Gravies, sauces*a</td>
<td>4.0</td>
</tr>
<tr>
<td>Dairy products analogs*a</td>
<td>0.5</td>
</tr>
<tr>
<td>Hard candy</td>
<td>0.4</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>3.8</td>
</tr>
<tr>
<td>Seasonings and flavors<em>a</em>b</td>
<td>1050.0</td>
</tr>
</tbody>
</table>

*a Only one, two, or three firms reported using BHA in any product included in the food category.

*b The level of 1050.0 ppm is based on the use of BHA in flavor and spice blends sold to food processors in bulk. When used for seasoning or flavoring the BHA concentration would be diluted several hundred-fold in the food as consumed. Because fewer than four firms reported addition of BHA in this category, it is probable that most foods in this category do not contain BHA.
TABLE II
Possible Average Daily Intake of Butylated Hydroxyanisole by Age Group (9)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Intake mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5 mo</td>
<td>0.6</td>
</tr>
<tr>
<td>6-11 mo</td>
<td>6.4</td>
</tr>
<tr>
<td>12-23 mo</td>
<td>8.0</td>
</tr>
<tr>
<td>2-65+ yr</td>
<td>12.7</td>
</tr>
</tbody>
</table>

TABLE III
Consumption of Butylated Hydroxyanisole Based on Total Quantity Used Annually in Foods (9)

<table>
<thead>
<tr>
<th>Relative amounts used&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total used (1970)&lt;sup&gt;b&lt;/sup&gt; kg</th>
<th>Per capita daily intake&lt;sup&gt;c&lt;/sup&gt; mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970/1960</td>
<td>418,000</td>
<td>5.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based only on the reports from those respondents to the National Research Council (NRC) survey who submitted information for both 1960 and 1970 (9).

<sup>b</sup>Total usage is based on the sum of the kilograms used in foods as supplied by NRC and Flavor and Extract Manufacturers' Association (FEMA) recalculated to 100 percent from survey data that the NRC subcommittee estimated to represent about 60 percent of the actual usage.

<sup>c</sup>Based on a U.S. population of 205 million.

The Joint FAO/WHO Expert Committee on Food Additives has suggested that 0 to 0.5 mg per kg body weight of BHA, BHT, or the sum of both would be an acceptable daily intake for man (4). In its evaluation of BHT, the Select Committee estimated that the average daily intake for an adult was 0.2 mg per kg (10). These estimates of the Select Committee for BHA and BHT would be 0.3 to 0.4 mg per kg body weight in toto; this figure is within the range considered acceptable by the FAO/WHO Expert Committee.
Johnson (11) calculated the maximum probable BHA intake for an average adult male based on a daily intake of 3000 kcal. The United States diet is approximately 40 percent fat on a caloric basis, or 10 percent fat by weight (133 g). Johnson (11) estimated 10 percent of the fat would contain added antioxidants (about 15 g). With 0.02 percent as the maximum allowable level of addition (2), the daily intake of a 60 kg man would be 3 mg or 0.05 mg per kg body weight per day. The Select Committee believes this calculation may be more reliable than the calculated intake figures presented in Table II and III.
IV. BIOLOGICAL STUDIES

Absorption and metabolism

There is considerable evidence that BHA is absorbed from the gastrointestinal tract and metabolized by several animal species. In one study, Astill et al. (12) gave a single oral dose of 400 mg per kg of BHA to groups of four or eight male and female Sprague-Dawley albino rats and studied urinary excretion over a 5-day period. Approximately 5 percent of the dose was excreted unchanged in the urine, while 61 to 82 percent was excreted as BHA glucuronide and 11 to 16 percent as the ethereal sulfate. Recovery of doses from 0.002 to 0.1 g per kg ranged from 81 to 100 percent. For single oral doses of 400 mg per kg of 2-tert-butyl-4-hydroxyanisole, 72 percent of the dose was excreted as ethereal sulfate in 5 days. In contrast, the 3-tert-butyl isomer was metabolized similarly to the commercial BHA mixture, 57 to 71 percent of the dose being excreted as glucuronide in 5 days. Administration of several successive daily doses of 500 mg per kg of BHA decreased the percent recovery of the conjugates. Approximately 5 percent of the administered dose appeared in the urine as free BHA and less than 1 percent was found in the feces. The possibility of 0-demethylation of the 2-isomer as a minor metabolic pathway was recognized.

Dacre (13) reported that rats excreted 90 percent of a single oral dose (80 to 100 mg) of BHA in the urine conjugated with glucuronic acid. Golder et al. (14) found excretion of 97 µg of tritiated BHA given to albino rats (sex and strain not specified) by intraperitoneal injections to be as the conjugated derivative at 86, 89, and 91 percent in 24, 48, and 96 hours, respectively.

After receiving 1.0 g of BHA in olive oil by stomach tube (dosage approximately 0.5 g per kg), rabbits excreted 46 percent of the solution as glucuronides, 9 percent as ethereal sulfates, and 6 percent as free phenols. The recovery ratios for the glucuronides and ethereal sulfates on a 0.25 g per kg dose were 60 and 12 percent; on a 0.125 g per kg dose, 84 and 18 percent, respectively (15).

Fasting dogs given doses of 50 and 250 mg per kg of BHA had increased amounts of ethereal sulfate and glucuronides in the urine (16). In three dogs given doses of 350 mg per kg in lard mixed with their diet, 60 percent of the BHA was excreted unchanged in the feces in 3 days (17). The remainder was found in the urine, largely as ethereal sulfate (23 percent), tert-butyl hydroquinone glucuronide (5.5 percent), free BHA (3.6 percent), and an unidentified phenol.

Six adult men were given a single oral dose of BHA (0.4 to 0.7 mg per kg) by capsule, or 0.5 mg per kg by olive oil-milk emulsion (17). Less
than 1 percent of the administered doses appeared in the urine as free BHA or as ethereal sulfates. The time for excretion of the administered doses varied between 27 and 50 hours.

A single oral dose of 40 mg $^{14}$C-labeled BHA, approximately 0.5 mg per kg, was administered to two men (18). In 2 days 60 to 73 percent of the radioactivity appeared in the urine; within 11 days, 80 to 87 percent appeared in the urine. It was suggested that tissue storage accounted for the fraction of the dose not excreted.

**Acute toxicity**

When given by gavage to rats, BHA had an LD$_{50}$ value in excess of 2.0 g per kg body weight; similarly, when given to mice, the observed LD$_{50}$ value was 1.25 to 2.0 g per kg (19-21). Hiraga et al. (22) have reported LD$_{50}$ values of 2.0 g per kg body weight for male and female rats following oral administration of BHA. The LD$_{50}$ for BHA in the rat was 4.1 g per kg when the compound was dissolved in corn oil and in excess of 5 g per kg body weight when given in water emulsion to a fasted rat (23). Daily doses (by gavage) of 2 g per kg body weight of BHA were lethal to rabbits in 3 to 7 days, and daily 1.0 g per kg body weight doses were lethal to rabbits in 11 days (15).

**Short-term studies**

Eight rats (strain unreported) receiving a total oral dose of 5.0 to 7.0 g of BHA over a 2.5 month period (total dose estimated at 600 mg per kg) exhibited a lag in weight gain compared to that of the control group (19). This effect was more pronounced in the first month. Blood catalase and peroxidase activities were reduced. Increases in liver weights and body fat content were evident, but no pathological differences were observed between experimental and control animals at autopsy.

Weanling rats (strain and number unreported) were fed diets containing up to 1 g per kg body weight BHA for 6 months. Weight gains were reduced in animals fed the diets containing the highest dosage levels, but no pathological effects were noted at the end of the experiment in any animals fed BHA, even at the highest level (23).

Graham and colleagues (24, 25) used Tenox II® (a commercial product containing 20 percent BHA, 6 percent propyl gallate, 4 percent citric acid and 70 percent propylene glycol) as a source of antioxidants in two feeding trials with Wistar albino rats. Using four groups of 26 and 15 males and equal numbers of females for 52 or 32 weeks, respectively, they fed the animals a diet containing 75 percent bread with Tenox II® added at level of about 2.7 mg of BHA per kg in the total diet (about 50 times the normal usage). The bread also contained chlorine dioxide, sodium propionate and polyoxyethylene monostearate. They observed no deleterious effects on
growth and survival of either males or females in either study. Histopathologic examinations of the major organs and tissues revealed no changes that could be attributed to the antioxidants.

When young male rats were fed 0.1 or 0.25 percent BHA (approximately 100 to 250 mg per kg body weight), liver weight, biphenyl-4-hydroxylase, and 4-methoxybiphenyl demethylase activities were not affected (26). BHA fed for 9 days at 0.5 percent likewise did not affect liver weight significantly; however, a significant increase in biphenyl-4-hydroxylase activity was observed. Martin and Gilbert (27) reported that rats given 500 mg per kg BHA daily for 21 days by stomach tube exhibited increased liver weights within 24 hours of the initial dose. Liver weight remained elevated for the experimental period. In addition, increases in biphenyl-4-hydroxylase and BHT-oxidase activities, and in urinary ascorbic acid excretion were noted during the experimental period. When similar doses of BHA were incorporated in the diet, liver weight increased and enhanced enzyme activities occurred, but at a less rapid rate.

BHA was administered to male and female SPF Carworth rats by stomach tube daily for 1 week at levels of 50, 100, 200 and 500 mg per kg body weight (28). No changes in liver fat were observed; however, significant increases in liver weights were noted in males at doses of 100, 200 and 500 mg per kg but in females only at 200 and 500 mg per kg. Gilbert and Golberg (29) observed that in rats (sex and strain not specified) receiving oral doses of 500 mg per kg twice a day, both relative liver weight and urinary ascorbate level were elevated but returned to normal by the sixth day of treatment. Continued administration for 12 weeks resulted in liver enlargement, but there was no discernible alteration of liver enzyme activities. In a related study, BHA dissolved in peanut oil was administered to adult female rats (Carworth Farm SPF strain) at 500 mg per kg for a period of 84 days (30). This experimental feeding trial included animals that were pregnant at some time during the 84 day period. Moderate but usually statistically insignificant increases (up to 10 percent) in relative liver weight and liver protein were observed. The activities of the liver enzymes — hexobarbital oxidase, nitroanisole demethylase, codeine demethylase and aminopyrine demethylase — were not affected. The authors concluded that it is probably appropriate to disregard hyperfunctional liver enlargement in assessing the acceptability of BHA as a food ingredient.

Gaunt et al. (31) fed BHA to weanling male and female Carworth SPF rats at a level of 0.1 percent of the diet (50 mg per kg) for periods of up to 16 weeks. There were no effects on growth; however, a significant but minor increase in urinary ascorbic acid excretion was observed at 4 weeks. There was a small increase in relative liver weight at 4 weeks but this was not observed thereafter. In a few instances increases in adrenal weight were seen in females.

In a subsequent study Gilbert et al. (32) found the livers of female albino (CFE strain) rats given 150 mg per kg per day of BHA for 7 days
weighed 11 percent more than those of control animals. Supernatants of
the liver homogenates showed an increase in biphenyl-4-hydroxylase activity
over the controls but no increase in hexobarbitone oxidase activity. The
authors concluded from similar studies with 36 phenols that liver enlargement
was not invariably related to the induction of either drug metabolizing activities
or to uridine diphosphate glucose dehydrogenase. The conclusion has been
supported by Crampton et al. (33) who fed diets containing 0.4 percent BHT
(about 200 mg per kg body weight) to female rats for 80 weeks. There was an
increase in relative liver weight and marked enhancement of the activities
of ethylmorphine N-demethylase, alanine 4-hydroxylase, biphenyl-4-hydroxy-
lase and NADPH-cytochrome c reductase and the contents of cytochromes
P-450, b5 and microsomal protein. The changes persisted until the treatment
was stopped after 80 weeks. Morphological changes observed were centri-
lobular cell enlargement and hypertrophy of the smooth endoplasmic reticulum.
There was also depression of glucose 6-phosphatase. Crampton et al.
concluded that these results, support in general, the concept that liver en-
largement accompanied by induction of drug-metabolizing enzymes represents
an adaptive response.

Eighteen beagle dogs fed BHA at dose levels of 0.3 to 100 mg per kg per
day for 1 year remained in good health throughout the period (34). Upon
histologic examination, there was no evidence of tissue change and no trace
of BHA in adipose tissue, brain, liver or kidney. In a comparable study,
BHA was fed daily to weanling cocker spaniel pups at levels of 5, 50 and 250
mg per kg body weight for 15 months (16). The weight gains were normal
except for those animals receiving the highest dosage. In three of four dogs
fed 250 mg per kg body weight (a rate that is 1110 times the maximum level
allowed in fat for human consumption by GRAS regulations), liver parenchymal
degeneration was noted as well as diffuse granulocytic infiltration, with marked
narrowing of the hepatic sinusoids. Kupfer cells contained an increased amount
of hemosiderin. There was also focal accumulation of bile pigment in periportal
areas. The fourth dog in this experimental group consistently ate only half of
the ration and did not exhibit liver injury. Wilder et al. (16) noted that the
three dogs fed BHA at the highest level consumed more than 1.5 g of BHA per
day during the 15-month feeding period and the fourth dog about 786 mg per day
(183 mg per kg body weight). They concluded that dogs may ingest BHA at
levels of 220 times the maximum allowable level for the antioxidant in lard
for periods of 15 months without harm. Dogs given daily doses of BHA com-
prising 0.3 percent of their diet (100 mg per kg) for 1 year had virtually no
storage of BHA in brain, adipose tissues, liver, or kidneys (34).

Six pigs (breed and sex not reported) were given 0.1 percent BHA
(approximately 40 mg per kg body weight) in their diet for over 4 months
(35). Analysis of fatty tissues revealed no antioxidant in fatback, pork fat,
muscle, liver, kidney, or blood. Similar results were obtained when chickens
were fed 0.1 percent BHA (dose estimated to be about 125 mg per kg body
weight) in their diet for 8 weeks (35).
Three 1-month-old and three sexually mature rhesus monkeys were given BHA at 500 mg per kg in corn oil by gastric intubation daily for 4 weeks; two juveniles were given BHA at 50 mg per kg under the same regimen (36). BHA in daily doses of 500 mg per kg caused significant increases in liver weights at 4 weeks while lesser increases seen in animals receiving 50 mg per kg were of questionable significance. Histopathological investigations of all organs failed to reveal major changes that could be related to ingestion of BHA. Corn oil alone produced slight to moderate increase in the number of lipid droplets within the cytoplasm of the hepatic cells. There were also indications of hepatocytomegaly and enlargement of cell nuclei in juvenile animals receiving BHA at the higher level. Hepatocytes showed proliferation of the endoplasmic reticulum. In addition, there was fragmentation of the nucleolus in 15 percent of the hepatic cells in livers of animals receiving 500 mg per kg. Other nuclear changes included development of long, coarse, randomly dispersed nucleolar fibrils. An apparent increase in nitroanisole demethylase and decrease in glucose-6-phosphatase were noted and considered significant. Total cellular RNA was not significantly altered and histological evaluation of all other organs failed to show any pathological changes attributable to BHA. None of the above noted changes was observed in animals receiving 50 mg per kg for 28 days. It is noteworthy that in contrast to observations in rats, BHA had a greater effect on certain aspects of metabolism than BHT in these studies on monkeys. Allen and Engblom (36) concluded that it seemed reasonable to assume BHT and BHA metabolism are similar in man and monkeys but may be different in the rat. However, they indicated the effects observed in these studies, as with previous studies, were related to metabolites of BHT or BHA rather than the parent compounds.

In a subsequent report from this laboratory lipid changes in blood and livers of the same animals were described (37). Few statistically significant alterations in lipid levels were observed. At 500 mg per kg, BHA seems to counteract corn oil effects. Decreased plasma and liver cholesterol levels were observed. At 50 mg per kg only liver cholesterol was lowered. The investigators suspected a relationship between large doses of BHA and BHT, the level of dietary vitamin E and the type and level of dietary lipid with respect to their role in lipid metabolism in primates.

**Long-term studies**

Albino rats were fed BHA in lard at levels of 0.01 and 0.1 percent (calculated to be 5 and 50 mg per kg body weight) for 2 years and Norway hooded rats were fed BHA in lard at levels of 0.004, 0.01, and 0.5 percent, (approximately 0.2, 5.0, and 250.0 mg per kg body weight) respectively, for 8 months (38). Only at the 0.5 percent level was there a small but significant reduction in mature weight and an increase in relative liver weight. There were no effects on the reproductive cycle, histology of the spleen, kidney, liver or skin as well as no effects on heart, spleen or kidney weights.
at this dose or at lower dose levels tested. Heating the lard containing BHA to 150°C for 30 minutes eliminated the effects on body weight and relative liver weight at maturity seen in animals fed at the 0.5 percent level. Brown et al. (38) attributed these differences to possible loss of BHA by volatilization during heating.

In these studies, no significant differences were found in the number or average weight of pups born to females during the 8-month experimental period. Volkova (39) observed no adverse effects on sex cycle phases, or on histology of endocrine glands of albino rats or guinea pigs fed BHA at 0.4 mg per kg body weight for 6 months. In a 2-year feeding study, Brown et al. (38) noted no adverse effects on reproduction of Norway hooded or albino rats fed 5.0 or 250.0 mg per kg body weight BHA in the diet.

Telford et al. (40) found that a total dose of 0.5 g BHA added to the diet of pregnant 200 g Walter Reed-Carworth Farm rats during a 21-day period after mating, decreased the fetal resorption rates. The authors termed this response "a beneficial effect." In contrast to the above results, Daniyalov (20) reported that female rats (strain unreported) fed mixtures of BHA and propyl gallate at doses as low as 10 to 20 mg per kg, respectively, for up to 9 months, failed to produce progeny when mated with males fed similarly. No explanation for these observations was provided by the investigator.

Allen (41) incorporated BHA and BHT into the diet of six adult rhesus female monkeys for a period of 2 years. The amounts added were adjusted to ensure a daily intake of 100 mg per kg of body weight (50 mg BHA and 50 mg BHT). Following the initial year of exposure, during which the monkeys experienced no clinical abnormalities attributable to the antioxidants, they were bred to rhesus males that had received no BHA or BHT. The gestations were free of complications and normal infants were delivered naturally. Growth rate, hemograms and behavior of the infants were similar to those of control infants. The adults and infants were evaluated for 2 years following the administration of the antioxidants. During this period the adult females continued to have normal infants and the infants born during the exposure period remained healthy.

Mutagenicity

BHA did not induce mutations in the host-mediated assay with Salmonella typhimurium or Saccharomyces cerevisiae, or in the dominant lethal assay in rats at doses of 15, 150, and 1500 mg per kg when given by intubation in isopropyl alcohol. Cytogenetic tests with rat bone marrow metaphase chromosomes and human embryonic lung tissue cultures with doses of 2, 20, and 200 μg BHA per ml revealed no significant chromosomal aberrations (42). In addition, in vitro studies of BHA at a dose equivalent to 750 μg per kg using S. typhimurium and S. cerevisiae, both unactivated and activated with
mouse, rat, or monkey tissue homogenates, failed to show mutagenic activity (43). Joner (44) obtained similar results with an analogous assay system using S-9 homogenate prepared 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.

Teratogenicity

In an extensive study of teratogenic effects, Clegg (45) administered BHA in peanut oil by oral intubation to female Imperial Chemical Industries SPF mice, and females of four rat strains (Tuck, Carworth SPF, Porton albino, and Benger hooded). He used doses of 500, 750, and 1000 mg per kg by several regimens including single massive doses on a specific day of gestation, repeated daily doses from mating through pregnancy, and daily doses for periods of 7 weeks prior to mating and continuing through mating and gestation. He noted that at all doses BHA 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 at the dosage levels and regimens employed in these investigations.

Reid (46) reported that BHA at doses of 1.0 and 2.5 mg per kg injected into the fertilized egg prior to incubation increased the occurrence of head and limb abnormalities in the avian embryo teratology test. However, other studies indicate that the administration of oral doses of BHA to mice, rats, or hamsters during pregnancy had no observable teratogenic effects or effects on nidation or on maternal or fetal survival (47). Mice given doses up to 225 mg per kg body weight by oral intubation from day 6 to day 15 of pregnancy did not exhibit abnormalities in either soft or skeletal tissues that differed significantly from the number that occurred spontaneously in sham-treated controls. Similar observations were reported for pregnant rats receiving doses up to 200 mg per kg body weight from day 6 through day 15, and for hamsters receiving doses of 120 mg per kg body weight from day 6 through day 10 of pregnancy (47).

Carcinogenicity

Several studies have included long-term feeding trials and subsequent histopathological examination of most body tissues and organs (16,23,24,38). In all cases, no carcinogenic effects of BHA were reported. Hodge et al. (48) used BHA as a noncarcinogenic control substance in a study of tests for evaluating chemical carcinogenesis in mice. No evidence for carcinogenicity was observed in six groups of 50 male and 50 female mice (C3H/Anf strain) each given single subcutaneous injections of 10 mg BHA in trioctanoin during an observational period of 273 to 575 days. When equivalent groups were given weekly skin applications of 0.1 mg or 10 mg BHA in acetone, there was no gross or microscopic evidence of tumor formation in the skin of these mice after 459 to 519 days.
Riley and Seal (49) reported that daily topical application of 20 percent BHA in lanolin to the ears of guinea pigs for a period of 2 to 6 weeks produced morphological lesions consisting of intrusion of pseudopodia from epithelial cells through defective areas in the basement membrane, a phenomenon termed "microinvasion" which the authors noted as a phenomenon associated with neoplastic change in the epithelial cells and hence might be interpreted as an evidence of carcinogenesis. No further effects were observed. Grasso and Rostron (50) have suggested that the epidermal pseudopods induced by prolonged application of BHA are merely the ultrastructural counterpart of the initial proliferative activity induced by application of a carcinogen or a simple irritant. Referring to previous work by other investigators, Grasso and Rostron (50) suggested that BHA was a simple irritant and when topical application ceased, lesions would be reversible.

Berry et al. (51) applied 1 mg BHA in acetone solution topically to the shaved backs of 30 6- to 8-week-old female CD1 Charles River mice twice weekly for 30 weeks. Weekly observations and histological examination at the conclusion of the experiment revealed no papillomas or carcinomas. Completely negative results were also obtained in another group of 30 mice, treated similarly, after a 1-week initiation period with topically applied 7,12-dimethylbenz(a)anthracene.

In a study of several food additives and chemotherapeutic agents, Stoner et al. (52) reported that intraperitoneal injection of a total dose of 1.2 and 6.0 g per kg of BHA in male and female A/He mice over 24 weeks failed to produce any tissue abnormalities or pulmonary tumors that could be attributed to the BHA injection.

Wattenberg (53) noted that BHA in the diet for 18 weeks at 5 or 10 mg per g of diet (estimated as 1 to 2 g of BHA per kg per day) inhibited polycyclic hydrocarbon-induced carcinogenesis in the forestomach of the mouse (females of Ha/ICR and A/HeJ strains) and in the mammary gland of the female Sprague-Dawley rat. In a subsequent study with BHA added at 5 mg per g of chow, (ingestion level about 20 mg BHA per mouse per day) female A/HeJ mice were protected from chemical carcinogen-induced pulmonary adenoma (54). Both Wattenberg (54, 55) and Cumming and Walton (56) suggested that the protective action of BHA is related to induction of drug metabolizing enzymes in liver microsomes. Cumming and Walton (56) observed that 10- to 12-week-old (C3H X 101) F₁ hybrid mice fed laboratory meal containing 1 percent BHA ad libitum for 30 days survived 30 days longer than the control animals following a single intraperitoneal injection of 25 mg per kg of the mutagenic and carcinogenic agent, ethyl methanesulfonate. Lam and Wattenberg (57) concluded that BHA inhibited carcinogenesis by benzo(a)pyrene in mice given 5 mg of BHA per g of diet by
decreasing the amount of epoxidation of the hydrocarbon and by increasing the amount of 3-hydroxybenzo(a)pyrene, a presumed detoxification product.

Behavioral effects

Stokes et al. (58) reported that 0.5 percent BHA or BHT in the diet of pregnant mice (Camm Research Laboratory) for 30 days (dose approximately 750 mg per kg body weight per day) resulted in brain enzyme changes in the newborn offspring including a 50 percent decreased activity in brain cholinesterase and changes in isozymes as well. In addition, the behavior of animals receiving either BHA or BHT in the diet differed from control animals with respect to sleep, aggression and freezing behavior, a marked decrease in the exploratory reflex and a reduction in weight.

In a subsequent study, Stokes and Scudder (59) reported altered behavioral patterns in Swiss-Webster mice reared on diets containing 0.5 percent by weight Tenox Food Grade BHA (Eastman Organic Chemicals). Dams received the ration during pregnancy and weaning; pups received it during weaning and early growth until 6 to 7 weeks of age. Tenox Food Grade BHA at 0.5 percent of the diet is equivalent to approximately 750 mg per kg body weight per day. Compared to controls, no significant differences in digging, freezing, carrying, grooming, or sexual behavior were noted. However, BHA-treated animals did exhibit increased nest exploration behavior and decreased sleeping, self-grooming, learning rates and orientation reflexes. The authors speculated that BHA could affect the normal sequence of neurological development in the young animals.

Allergenicity

Fisherman and Cohen (60) reported that seven patients suspected of BHA and BHT sensitivity exhibited several signs and symptoms of allergy when challenged with oral doses of 2.1 to 4.2 mg per kg body weight of either BHA or BHT after 12 hours of fasting. Manifestations included chronic nasal blockage, frequent nasal polyps, chronic vasomotor rhinitis, headaches and associated asthma with occasional marked diaphoresis, somnolence, high retrosternal pain radiating to the back, flushing and suffusion of the conjunctiva. Altered bleeding times occurred in BHA- and BHT-sensitive and in iodide-sensitive but not in aspirin-sensitive patients or control subjects.

Cloninger and Novey (61) attempted to reproduce this study with seven patients exhibiting allergic rhinitis and asthma of unknown etiology. None of these seven patients had an exacerbation of respiratory disease
when challenged with oral doses of about 5 to 14.2 mg per kg of either BHA or BHT. They noted 100 to 200 percent variation in bleeding times during control periods, and were unable to reproduce the changes in bleeding times noted by Fisherman and Cohen (60). Based on their clinical studies, Cloninger and Novey (61) questioned the validity of BHA intolerance as a clinical entity.

Nevertheless, Fisherman et al. (62) have reported unrelated studies on lipoprotein patterns in which 37 patients were identified as BHA or BHT intolerant. Roed-Petersen and Hjorth (63) observed four of 112 patients tested over a 3-year period who exhibited positive patch tests to BHA or BHT. Two were sensitive to both antioxidants and one was sensitive to BHA only. Two of these four patients had acute flares of vesicular eczema after oral administration of 10 mg each of BHA and BHT. Meneghini et al. (64) found one out of 360 patients with eczematous dermatitis who reacted to topical application (patch test) of 5 percent BHA in petroleum ether.

Effects on liver enzymes

Results of a number of studies indicate that orally administered BHA affects activity of several liver microsomal enzymes (26, 27, 32, 33, 36). However, quantitative changes are apparently related to dosage and duration of administration because Creaven et al. (26) and Gilbert and Golberg (30) have noted the absence of stimulation under certain conditions. For example, Martin and Gilbert (27) observed that rats given 500 mg per kg BHA daily for 21 days by stomach tube exhibited increased activity of the liver mixed function oxidase, biphenyl-4-hydroxylase, while Creaven et al. (26) observed no increase in activity of this enzyme in rats fed BHA at 100 to 250 mg per kg. Creaven et al. (26) did observe increased biphenyl-4-hydroxylase activity in livers of rats fed 500 mg per kg BHA for 9 days.

Vainio (65) studied the effects of BHA and BHT in vitro on microsomal cell fractions from livers of male Wistar rats. Both antioxidants at 0.02 mM inhibited enzymatically-induced lipid peroxidation and consequently, the decrease in microsomal monooxygenase and increase in UDPglucuronosyltransferase activity caused by the peroxidation of the membrane lipids. At higher concentrations (i.e. 10 mM) BHA totally inhibited UDPglucuronosyltransferase activity and microsomal monooxygenase activity. Yang et al. (66) found that both BHA and BHT inhibited competitively in a rat microsomal system from rats pretreated with methylcholanthrene. Both antioxidants bind to cytochrome P-450 and do not inhibit reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase activity. However, a NADPH-lipid peroxidase system was inhibited 90 percent by 4 μg BHA.
Liver microsomes of A/HeJ female mice fed BHA in the diet at 5 mg per g (dose estimated to be 75 mg per kg body weight) exhibited alterations of mixed function oxidase systems (57). The amount of cytochrome P-450 was increased and the quantitative production of epoxides of benzo(a)pyrene was reduced in liver extracts from mice fed BHA as compared to those of control animals. Lam and Wattenberg (57) concluded that BHA inhibited carcinogenesis of benzo(a)pyrene by increasing its detoxification and decreasing its activation as a result of the effects on mixed oxidase enzyme systems. In related studies, Speier and Wattenberg (67) concluded that liver microsomes from A/HeJ mice fed 5 mg per g (dose estimated to be 75 mg per kg body weight) exhibited no change in aryl hydrocarbon hydroxylase activity, but a decrease in binding of benzo(a)pyrene metabolites to DNA.

Sporn and Dinu (68) found that liver homogenates from young Wistar rats fed 0.01 to 0.1 percent BHA in the diet for 8 weeks (dose estimated to be 10 to 100 mg per kg body weight) had reduced oxygen uptake with succinate as a substrate while rats fed similarly without succinate had increased oxygen uptake and altered oxidative phosphorylation. However, Feuer et al. (28) noted BHA intubated at doses up to 500 mg per kg into the stomachs of male and female SPF Carworth rats produced no changes in activities of liver glucose-6-phosphatase or glucose-6-phosphate dehydrogenase. Daniyalov (20) reported statistically significant decreases in blood sugar cholinesterase, catalase, and ketone bodies in rats fed 100 mg of BHA per kg body weight and propyl gallate at 50 mg per kg.

Karplyuk (69) reported that blood catalase and peroxidase activities were reduced when rats were fed BHA at approximately 600 mg per kg body weight. Significant increases in oxygen uptake were detected in liver homogenates from Wistar rats fed BHA over an 8-week period at levels estimated to be up to 100 mg per kg body weight (67). In a similar study, rats fed BHA at about 400 mg per kg body weight in dry food for 4 and 8 weeks had no change in their blood characteristics except for a significant decrease in leucocyte phagocytic activity (70).

Mice fed BHA at 4 mg per kg body weight for 30 to 35 days exhibited no differences from control animals with respect to intestinal enterokinase and alkaline phosphatase as well as pancreatic amylase and lipase activities (69).

The effects reported on liver microsomal enzymes of monkeys differ significantly from effects reported on these enzymes from rodent livers. Oral doses of BHA at 500 mg per kg per day for 28 days produced in juvenile monkeys a decrease in microsomal glucose-6-phosphatase and an increase in hepatic nitroanisole demethylase (36). Differing levels of demethylase induction were found in infant and juvenile monkeys. The effects of 50 mg per
kg per day on these enzymes were not significant. Slight decreases in liver succinic dehydrogenase, blood catalase and serum acid phosphatase in monkeys given 500 mg per kg body weight BHA were not significant. Cytochrome P-450 content of hepatic microsomes was not altered following BHA doses of 500 mg per kg body weight.

Interaction with vitamin E and lipids

Interactions between naturally occurring and added antioxidants have been assumed to occur in foods. Substances possessing vitamin E activity, such as α-tocopherol, also have antioxidant properties. There is considerable evidence that the presence of synthetic antioxidants may reduce the occurrence of the characteristic signs of vitamin E deficiency in several animal species or in their progeny fed vitamin E deficient diets (11, 67). Pascal (71) has suggested that the role of vitamin E in vivo may not be related directly to lipid peroxidation, but indirectly by modification of the intestinal barrier, thereby preventing absorption of exogenous lipid peroxides.

Branen (72) concluded that long-term chronic ingestion of BHA and BHT may be beneficial in sparing vitamin E. Alfin-Slater (73) and Krishnamurthy and Bieri (74) have pointed out that certain other antioxidants will prevent development of vitamin E deficiency in various animal species. At levels of use in human food (up to 0.2 percent of fat and oil content) and experimental dosages up to 500 times higher, BHA and BHT are effective in preventing dietary reticulocytosis (75) and encephalomalacia (76) in chickens; reducing the frequency of fetal reabsorptions (40) and preventing oxidation of perirenal fat in the rat; and preventing oxidation of chicken and pig fat in vivo (35). However, Wilson and Hartroft (77) observed BHA or BHT had no effect on prevention of myocardial infarction in rats fed diets with or without vitamin E added.

Thus there is evidence of interactions between vitamin E and several antioxidants including BHA; however, the nature of these interactions remains to be established.

Feeding 4 mg per kg of BHA in lard to rats for 30 to 35 days had no influence on certain aspects of fat metabolism or on weight gain by the animals (78). No changes in liver glycogen, cholesterol, phospholipids, and concentration of liver fat or its iodine number were noted.

Effects on hormones

Posati and Pallansch (79) have reported that BHA suppressed the contractile response to bradykinin elicited in the smooth muscle of the guinea pig ileum by concentrations as low as 8 x 10^{-9} mole per liter. The threshold effect level for BHA in this in vitro test system was 0.87 μg per liter. Volkova
observed essentially no alteration of pituitary gonadotrophic hormone production in albino rats or guinea pigs fed BHA at a dosage of 0.4 mg per kg for 6 months.

Boehme and Branen (80) studied the synthesis of prostaglandins (PG) by microsomal fractions from bovine seminal vesicles. The presence of 6.7 μM BHA resulted in a 50 percent inhibition of PGE₁ synthesis while 3.08 μM BHA produced a 50 percent inhibition of PGE₂ formation. Based on calculated whole body concentrations of 1.06 μM BHA (191.1 µg per kg), 0.7 μM BHT (154.2 µg per kg), 1.1 μM ascorbic acid (193.7 µg per kg) and 1.15 μM tetrabutylhydroquinone (384.2 µg per kg), these authors concluded that low concentrations of food antioxidants could inhibit prostaglandin synthesis in vivo.

Miscellaneous studies

Fung et al. (81) have reported that BHA at 5 to 20 mg per plate inhibited growth, spore formation, pigmentation and aflatoxin production of Aspergillus flavus.

Fritsch et al. (82) studied the effects of BHA and other compounds on isolated cecal flora of rats. BHA at 400 µg per ml of incubation solution inhibited bacterial metabolism at a concentration which the authors considered close to that present in the alimentary tract. In another study, Fritsch et al. (83) found that at 2 mg per ml BHA reduced absorption of glucose and methionine by the perfused rat intestine. The toxicological significance of these observations is unclear.

Ford et al. (84) fed male rats 500 mg per kg per day of BHA or BHT and investigated the accumulation of p-aminobiphenyl (PAH) in kidney slices after one, two, four or six doses. PAH accumulation was depressed after one dose of either antioxidant. Increases in liver weights after the second dose were paralleled by increases in the PAH serum to slice ratio and approached normal after six doses. On addition of the antioxidants to kidney slices at concentrations known to depress metabolism of kidney cell cultures, BHA depressed accumulation of PAH to a greater extent than did BHT. The authors considered the difference in renal function which was observed between BHA and BHT to be due to the more rapid metabolism of BHA.

Comparison of BHA and BHT

A number of synthetic sterically hindered phenols have been developed for use as antioxidants. BHA and BHT are used similarly as antioxidants in foods particularly in fats and oils. Because of the similarities of their structures and antioxidant properties, several reviews have compared the
biological and toxicological properties of BHA and BHT (7, 11, 71, 72). Both antioxidants have been considered GRAS; however, in 1977, the FDA proposed deletion of BHT from the GRAS list and restriction of its use as a direct and indirect food additive under an interim food additive regulation (85).

In 1959 Brown et al. (38) reported results which stimulated a number of investigations on BHT. They found that 0.1 percent BHT in the diet with a 20 percent lard supplement reduced growth of Norway hooded male rats while no such effect was noted in male or female rats receiving diets with 0.1 percent BHT and a 10 percent lard supplement. BHA at 0.1 percent in the diet with 10 percent lard supplement had no effect on growth, while 0.1 percent BHA in a diet containing 10 percent hydrogenated coconut oil enhanced growth of male and female rats. BHT but not BHA at levels of 0.5 and 0.1 percent of the diet produced baldness and skin changes in Norway hooded rats but not in albino rats or mice. Three of 30 litters of rats which had been fed BHT contained anophthalmic young. Mating anophthalmic male and female litter mates while maintained on a diet of 0.5 percent BHT, however, failed to produce anophthalmic young. These findings of teratogenicity have not been confirmed in any other laboratory (11). Clegg (45) attributed the results reported by Brown et al. (38) to a vitamin A deficiency.

The biological effects of BHA and BHT have been studied in the same species and strains of rats under identical conditions (26, 28, 29, 31, 32, 44, 69, 72, 86, 87). These investigations suggest that, in general, BHA is less effective than BHT in producing liver hypertrophy in rats given equivalent dosages. This phenomenon is attributed to the more rapid transit of BHT through the body because of its metabolism. Both isomers of BHA are conjugated in man chiefly with glucuronic acid (17). Rabbits metabolize the compound similarly although small amounts are excreted as ethereal sulfates or free BHA (15). In the rat the 3-tert-butyl isomer is converted to the glucuronide while the 2-isomer is converted mostly to the ethereal sulfate (12). Dogs excrete BHA primarily as the ethereal sulfate although some tert-butyl hydroquinone was formed (17).

There is considerable evidence that the metabolism of BHT does not involve conjugation of the sterically hindered phenolic hydroxyl group. The principal avenue of metabolism in the rat is the oxidation of the 4-methyl or a butyl side chain (10). The hydroxylated derivatives also have antioxidant properties, e.g. 2, 6-di-tert-butyl-4-hydroxymethylphenol (IONOX 100®). These and the major metabolite, 3, 5-di-tert-butyl-4-hydroxybenzoic acid (BHT acid) are excreted free and as the ester glucuronide. Minor metabolites are a mercapturic acid, 3, 5-di-tert-butyl-4-hydroxybenzaldehyde and 1, 2-bis (3, 5-di-tert-butyl-4-hydroxyphenyl)-ethane. Daniel et al. (18) reported the principal metabolite of BHT in man to be the glucuronide of 4-carboxy-2-(1-carboxy-1-methylethyl)-6-(1-formyl-1-methylethyl)-phenol (BHT
dicarboxylic acid). Only minor quantities of free and conjugated BHT acid and traces of mercapturic acid were found. Allen and Engblom (36) concluded that metabolic products of BHT in monkeys were more closely related to those in man than to those of the rat.

Tissue storage due to lipid solubility may occur with both BHA and BHT; however, the amount stored is quite limited because of rapid metabolism and excretion. Several investigators have reported essentially no storage of free BHA in the rat, rabbit or dog (12, 15, 17, 34).

The enlargement of the liver and stimulation of drug-metabolizing enzymes of the microsomes, which have been observed with both BHA and BHT, are produced by at least 200 compounds which are of extremely diverse pharmacological activities (53, 56, 71, 88). Most authors agree that liver enlargement is an adaptive response which has been variously termed "work hypertrophy," "physiological overworking," or "hyperfunctional enlargement." At levels at which BHA and BHT induce liver hypertrophy, there is no evidence of persistent hepatotoxicity. The relative effects of BHA and BHT on rat liver enzyme systems in vitro and in vivo are likewise related to the level of the compound in the medium (27, 30, 32, 86). In general, alteration of microsomal enzyme activities require greater concentrations of BHA than those of BHT to produce similar results.

On the other hand, Allen and Engblom (36) and Allen (41) have reported that in the monkey BHA induces a pronounced increase in liver weight, proliferation of hepatic smooth endoplasmic reticulum, and an increase in hepatic microsomal enzyme activity and that these alterations with similar doses of BHT were less significant. Based on these data and results of similar studies in rats and man, these investigators concluded that both BHA and BHT were metabolized differently in rats and primates. Allen and Engblom (36) also reported differences in demethylase induction in infant and juvenile monkeys given either BHA or BHT. While no adverse effects on enzyme production, histopathological changes, liver hypertrophy or abnormal behavior were evident during the 2-year feeding trial, the data reported do indicate BHA had a greater effect than BHT on certain aspects of metabolism.

Similarly, Sgaragli et al. (80, 90) present data that suggest rat liver mitochondrial and lysosomal preparations exhibit a different sensitivity to BHA and BHT. At peroxidase-inhibiting concentrations, BHA caused release of larger quantities of proteins than BHT. While the two compounds dissociate acid phosphatase in equal measure, only BHA solubilized glutamic dehydrogenase. Sgaragli et al. (91, 92) also noted that compounds structurally similar to BHA are able to chelate protein-bound iron and suggested BHA might adversely affect red blood cell metabolism by binding to hemoglobin.
V. OPINION

The absorption and metabolism of BHA by several animal species and by man are well documented. Man excretes a minute quantity of BHA as the free phenol and an ethereal sulfate while the major portion is conjugated with glucuronic acid. In the rat, a larger percentage of administered doses is excreted as the free phenol and an ethereal sulfate. In rats, dogs, monkeys, and man, none of the metabolic products of BHA are antioxidants while in these species, metabolic products of BHT have antioxidant properties.

The acute toxicity (LD₅₀) of BHA is about 2 g per kg for mice, rats, and rabbits. The no-effect level for short-term effects in the rat has been estimated at 25 mg per kg. If this value should be approximately applicable to man, it is 125 to 500 times the estimated daily intake of 0.05 to 0.2 mg per kg. Doses of the order of 50 mg per kg or more when fed chronically to animals produced significant liver hypertrophy accompanied by proliferation of endoplasmic reticulum and a nonspecific stimulation of the synthesis of drug-metabolizing systems. Such effects disappeared on cessation of BHA intake but remained as long as the compound continued to be ingested. Two monkeys fed BHA at the 50 mg per kg level had marginal proliferation of the endoplasmic reticulum and stimulation of mixed function oxidases. It was noteworthy that at both the 50 mg and 500 mg per kg levels BHA had a more pronounced effect on liver weight in monkeys than BHT.

It is not clear that liver hypertrophy per se is a manifestation of microsomal enzyme induction. Liver hypertrophy is a gross measure of enzyme induction, but its absence may not necessarily mean that enzyme induction is absent. In addition, the findings from studies with rats, monkeys, and man suggest that BHA metabolism may vary among animal species.

Recent studies show that BHA action on enzyme systems in vitro occurs at very low concentrations. BHA can conceivably affect drug metabolizing enzymes in vivo which are the natural effectors of steroid hydroxylations and prostaglandin synthetases. In addition, there are reports that very low concentrations of BHA interfere with the action of bradykinin and prostaglandin synthesis. It would be desirable to determine whether BHA tissue levels resulting from chronic intake affect metabolic rates of natural substrates such as androgenic, progestational and adrenal steroids, pyridine nucleotides and cytochromes. In addition, the effects on metabolism of common drugs and oral contraceptives should be investigated.

In man single doses of BHA required 10 days for elimination probably due to the solubility and retention of the compound in fat. Thus, with a typical American diet which provides a regular intake of BHA, the chronic tissue level should be determined for man.
The evidence indicates that BHA is not mutagenic. While there are teratogenic effects of BHA in the avian embryo test system, several investigations using three mammalian species have failed to establish any teratogenic or embryotoxic potential when BHA is fed to young or adult and pregnant animals at dosages that greatly exceed estimates of human consumption. Data from several studies indicate that BHA is not a carcinogenic substance. There is evidence that BHA may interfere with synthesis of natural carcinogens and suppress or retard growth of tumors induced by known chemical carcinogens.

The Select Committee in its report on BHT identified areas of concern regarding the properties of BHT and indicated that additional studies are needed. Although the concentrations at which these responses occur are generally lower with BHA than with BHT, the qualitative effects are comparable. Concern was expressed about the possible enzyme inductive properties of BHT in extrahepatic tissues, particularly the intestine. Questions were also raised regarding the effect of induction of hepatic enzymes on the metabolism of steroids. The long-term effects of continuously maintained liver hypertrophy from multiple stimuli were also raised. This is a part of the general problem of adaptive responses of the liver which develop after the ingestion of many foreign substances including drugs, hormone analogues, insecticides, alkaloids, and carcinogenic polycyclic hydrocarbons.

While available data suggest that BHA in food is ingested at levels many times below that which produces short-term effects, several of these questions remain. The Select Committee concludes that studies on the tissue levels of BHA attained in man by chronic ingestion and the contribution of BHA to the general problem of enzyme induction should be assessed. Finally, chronic feeding studies with BHA at dosages equivalent to human exposure and use levels should be conducted in primates to determine the long-term effects of BHA on liver mixed function oxidase systems.

The Select Committee regards these questions as less urgent for BHA than for BHT and concludes that:

While no evidence in the available information on butylated hydroxyanisole (BHA) demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies be conducted.
VI. REFERENCES CITED


VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

Joseph F. Borzelleca, Ph. D., Professor of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Va.

Harry G. Day, Sc. D., Professor Emeritus of Chemistry, Indiana University, Bloomington, Ind.

Samuel J. Fomon, M. D., Professor of Pediatrics, College of Medicine, University of Iowa, Iowa City, Iowa.

Bert N. La Du, Jr., M. D., Ph. D., Professor and Chairman, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Mich.

John R. McCoy, V. M. D., Professor of Comparative Pathology, New Jersey College of Medicine and Dentistry, Rutgers Medical School, New Brunswick, N. J.

Sanford A. Miller, Ph. D., Professor of Nutritional Biochemistry, Massachusetts Institute of Technology, Cambridge, Mass.

Gabriel L. Plaa, Ph. D., Professor and Chairman, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

Michael B. Shimkin, M. D., Professor of Community Medicine and Oncology, School of Medicine, University of California, San Diego, La Jolla, Calif.

Ralph G. H. Siu, Ph. D., Consultant, Washington, D. C.

John L. Wood, Ph. D., Distinguished Service Professor, Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tenn.

George W. Irving, Jr., Ph. D., (Chairman), Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.
2. LSRO staff:

Kenneth D. Fisher, Ph.D., Director
Frederic R. Senti, Ph.D., Associate Director
C. Jelleff Carr, Ph.D., Director Emeritus
Richard G. Allison, Ph.D., Staff Scientist
Herman I. Chinn, Ph.D., Senior Staff Scientist
Andrew F. Freeman, Senior Staff Scientist
John M. Talbot, M.D., Senior Medical Consultant
Michael J. Wade, Ph.D., Staff Scientist

3. Ad hoc consultant:

Hanspeter Witschi, M.D., Assistant Professor, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

Report submitted by:

August 8, 1978
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances
PUBLIC HEARING ON BUTYLATED HYDROXYANISOLE,

HELD SEPTEMBER 26, 1977 *

Two requests for a hearing were received and the following individuals made presentations:

Dr. Bernard D. Astill, Health, Safety, and Human Factors Laboratory, Eastman Kodak Company, Rochester, N.Y.

Dr. James R. Allen, Food Research Institute, University of Wisconsin, Madison, Wis.

Northwest Food Processors Association, Cascade Plaza, Portland, Oregon 97201 (Request for hearing withdrawn.)
