# Toxicology and Biochemistry of Butylated Hydroxyanisole and Butylated Hydroxytoluene[1,2]

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## ABSTRACT

Butylated hydroxyanisole and butylated hydroxytoluene are used extensively as food antioxidants. It is estimated that man consumes ca. 0.1 mg/kg body wt daily of these antioxidants. At levels 500 times this level (50 mg/kg/day), both butylated hydroxyanisole and butylated hydroxytoluene appear to be free of any obviously injurious effects. However, at larger doses (500 mg/kg/day), both butylated hydroxyanisole and butylated hydroxytoluene result in certain pathological, enzyme, and lipid alterations in both rodents and monkeys, and butylated hydroxytoluene, in some cases, has been reported to have certain teratogenic and carcinogenic effects upon rodents. These alterations appear to differ markedly between rodents and monkeys, apparently as a result of differences which exist in the metabolism and excretion of butylated hydroxyanisole and butylated hydroxytoluene by these two species. However, in both animal species, the alterations appear to be physiological responses which are reversible upon removal of butylated hydroxyanisole and butylated hydroxytoluene from the diet. Long term chronic ingestion of butylated hydroxyanisole and butylated hydroxytoluene may be beneficial in sparing vitamin E and in modifying the acute toxicity of a number of mutagenic and carcinogenic chemicals.

## INTRODUCTION

Antioxidants are utilized widely as food additives. In 1967, 15.5 million lb antioxidants valued at $20.5 million were used by food manufacturers of the U.S. (1). These antioxidants help to maintain the quality of many food products by preventing oxidation of labile lipid components. It is estimated that the shelf-life of many food products can be increased 15-200% by the use of antioxidants (2).

Although many antioxidants are available, most antioxidant formulations contain butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Fig. 1). BHA and BHT are hindered phenolic compounds and prevent lipid oxidation by donation of a hydrogen to free-radicals. Commercial preparations of BHA are normally isomeric mixtures of 2-tert-butylated- and 3-tert-butylated-hydroxyanisole (4 and 96%, respectively).

Both BHA and BHT are considered generally recognized as safe for addition to food products. Food products covered by the Food, Drug, and Cosmetic Act are allowed to contain a total of 0.02% of BHA and BHT based upon the fat content of the food, while food products covered under the Meat Inspection Act and the Poultry Inspection Act generally can be treated with up to 0.01% of an individual antioxidant and a combined total of not more than 0.02% of all approved antioxidants based upon the wt of the fat (3). Exceptions to this tolerance exist, however, for certain prepared foods covered by special regulations (Table I), and both BHA and BHT can be used in packaging material as long as no more than 50 ppm of the antioxidants become part of the food (3).

Because of the widespread usage of BHA and BHT, it is essential that the safety of these antioxidants be evaluated critically. BHA and BHT have toxic effects at extremely high doses, both having an $LD_{50}$ of ca. 2000 mg/kg in most animals (4). This would appear to be of little consequence, however, since it has been estimated that man undoubtedly consumes less than 0.1 mg/kg/day of BHA and BHT (4,5). What is of concern, however, is the chronic toxicology and side effects of BHA and BHT. It is the purpose of this article to review some of the biochemical effects of subacute levels of BHA and BHT.

## PATHOLOGICAL EFFECTS OF BHA AND BHT

The major toxicological experiments with BHA and BHT have been limited to rodents, normally employing a dose of 500 mg/kg body wt (6-8). In rodents, hypertrophy of the liver has been the most common pathological change which occurred with administration of BHA and BHT, with BHT causing more severe effects than BHA. In most studies, BHA has resulted in only a moderate increase in liver wt (7), while BHT has resulted in marked hypertrophy of the liver (8) and a marked proliferation of the smooth endoplasmic reticulum (9,10).

Other changes reported include diminished initial growth rate and loss of hair of rats fed BHT (11), increased electrolyte and aldosterene excretion in rabbits fed BHA or BHT (12), and an enlargement of the adrenals and increased ascorbic acid output of rats fed BHA or BHT (7,13). Other workers have failed to observe such changes in their studies (7).

In contrast to the pathological changes noted in rodents, large doses of BHA caused more severe effects than BHT upon monkeys. Allen and Engblom (14) found no increase in relative liver size and only moderate proliferation of the smooth endoplasmic reticulum of infant and juvenile monkeys given 500 mg/kg/day of BHT for 1 month. The same dose of BHA, however, resulted in a pronounced increase in relative liver wt and a marked proliferation of hepatic smooth endoplasmic reticulum. Other differences in the responses of rodents and monkeys were observed ultrastructurally. Allen and Engblom (14) observed nucleolar fragmentation and the presence of large intranucleolar fibrils in many of the hepatic nuclei of monkeys fed 500 mg/kg body wt of either BHA or BHT. Neither Lane and Lieber (10) nor Botham, et al., (9) found any such

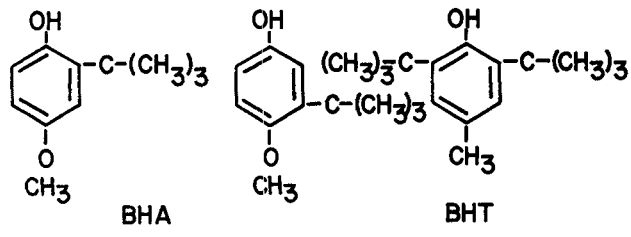


FIG. 1. Structures of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

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[2]Information paper, College of Agriculture Research Center, Washington State University, Pullman, Wash.

TABLE I

Addition Limits of Butylated Hydroxyanisole (BHA) and Butylated Hydroxytolune (BHT) to Various Foods Based upon Total Wt of Food[a]

| Food | BHA (ppm) | BHT (ppm) | Total permissible[b] (ppm) |
|---|---|---|---|
| Beverages and desserts prepared from dry mixes | 2 | --- | 2 |
| Cereals, dry breakfast | 50 | 50 | 50 |
| Chewing-gum-base | 1000 | 1000 | 1000 |
| Dry mixes for beverages and desserts | 90 | --- | 90 |
| Emulsion stabilizers for shortenings | 200 | 200 | 200 |
| Fruit, dry, glacéd | 32 | --- | 32 |
| Meats, dried | 100 | 100 | 100 |
| Potato flakes | 50 | 50 | 50 |
| Potato granules | 10 | 10 | 10 |
| Potato shreds, dehydrated | 50 | 50 | 50 |
| Rice, enriched | --- | 33 | 33 |
| Sausage, dry | 30 | 30 | 60 |
| Sausage, pork, fresh | 100 | 100 | 200[c] |
| Sweet potato flakes | 50 | 50 | 50 |
| Yeast, active dry | 1000 | --- | 1000 |

[a]Adapted from ref. 3.

[b]Combination of lawful antioxidant.

[c]Based upon fat content of sausage.

abnormalities in the hepatic nucleolus of rats fed BHT, although Lane and Lieber (10) did observe an increase in heterochromatin throughout the nucleus of rat hepatocytes during the first 3 days of BHT ingestion. No such changes have been reported for rats treated with BHA.

The above noted effects in both rodents and monkeys appear to be a result of a physiological adaptive response by the liver rather than a pathological change (8,14); however, according to Allen and Engblom (14), it is not clear when these changes cease to be normal and begin to represent toxic changes in the liver. Milner's work (15) indicated that BHT could have a toxic effect upon monkey cells. An in vitro dose of BHT decreased monkey kidney cell numbers and resulted in a rapid depression of cellular metabolism.

It is doubtful that man would ever be subjected to the high dosage levels necessary for the above noted effects. The effects are virtually absent in both rodents and primates treated with 50 or less mg/kg body wt of BHA or BHT (8,14). Also long term chronic studies of BHA and BHT have shown little or no effects upon rats (11, 16-18), dogs (19,20), or monkeys (21).

## CARCINOGENIC POTENTIAL OF BHA AND BHT

Information on the carcinogenic potential of BHA and BHT is quite limited. Hodge, et al., (22) reported that BHA did not have carcinogenic potential when fed to rats, mice, or dogs; however, other workers have reported that the nonbutylated form of BHA, 4-hydroxyanisole, caused basal cell encroachment to the dermis of guinea pigs (23) and epithelial hyperplasia (8) and hyperkeratosis in the hamster (24). Such changes often are observed in the development of experimental carcinomas.

Early studies had not indicated that BHT was carcinogenic; however, Clapp, et al., (25) recently found that, when mice were fed a diet containing 0.75% BHT, there was a marked hyperplasia of the hepatic bile ducts. These authors, however, were uncertain of the cause, pathogenesis, or fate of the observed lesion. Kamra (26) found that BHT was not mutagenic alone; however, it was an extremely effective radiosensitizer of $\gamma$-ray induced mutation in *Drosophila melagaster*.

Contrary to these reports, Ulland, et al., (27) and Cumming and Walton (28) have found that BHT decreased the mutagenicity and carcinogenicity of numerous chemical compounds. Shamburger (29) reported that BHA and BHT markedly reduced stomach cancer in mice. The mechanism by which BHA and BHT prevented the action of carcinogens is not known; however, BHT has been reported to increase detoxification metabolites of carcinogens (30) and to prevent carcinogen induced chromosomal breakage (31).

## EFFECTS OF BHA AND BHT UPON ENZYMES

Most of the enzyme changes occurring upon administration of BHA and BHT have occurred in the livers of experimental animals. In livers of rats, BHT caused the most extensive effects resulting in a decrease in glucose 6-phosphatase and acid phosphatase (6,32,33), an increase in a number of drug processing enzymes (8,9,34,35), and a decrease in cytochrome oxidase (36). BHA was reported to produce little or no effects upon microsomal enzyme activity (6,7,8,34,37). Sporn and Dinu (38) did find, however, that the livers of rats fed 0.01-0.1% BHA had reduced oxygen uptake with succinate as a substrate, while the livers of rats fed 0.1-1.0% BHA had increased oxygen uptake and altered oxidative phosphorylation. Some enzyme changes also resulted in the blood of rats fed BHA and BHT. BHA reportedly decreased plasma catalase and peroxidase, while BHT decreased plasma catalase, cholinesterase, and peroxidase (39).

Enzyme responses in monkeys differ significantly from those of rodents. In juvenile but not infant monkeys fed BHA at 500 mg/kg/day for 28 days, there was a pronounced decrease in microsomal glucose 6-phosphatase and an increase in hepatic nitroanisole demethylase (14). The same doses of BHT resulted in no change in glucose-6 phosphatase and only a moderate increase in hepatic nitroanisole demethylase. Dosage with 50 mg/kg/day of BHA or BHT resulted in a slight but insignificant increase in nitroanisole demethylase activity but had no effect upon glucose 6-phosphatase activity (14). Branen, et al; (40) reported a slight but insignificant decrease of liver succinic dehydrogenase, blood catalase, and serum acid phosphatase when monkeys were fed 500 mg/kg/day of BHA.

As with other changes occurring in the liver upon dosage with BHA and BHT, enzyme changes appear to be a normal physiological response and are reversible after return to normal diet (7).

## EFFECTS OF BHA AND BHT UPON LIPIDS

Although BHA has not been reported to alter lipid levels of rodents (41), numerous studies have indicated changes in

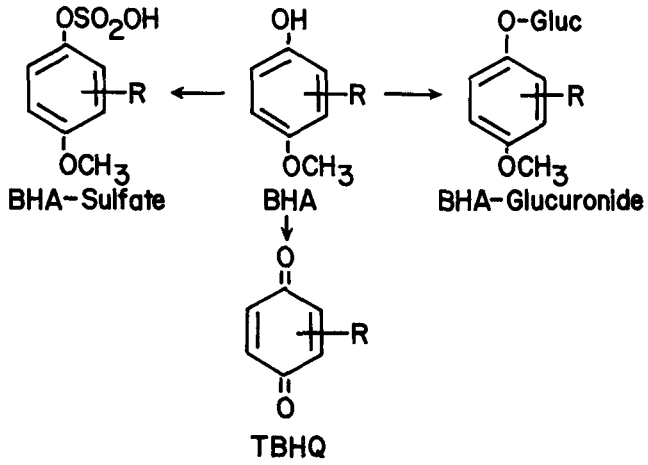


FIG. 2. Metabolic Fate of butylated hydroxyanisole. R represents tertiary-butyl group.

the blood and liver lipid levels of rodents dosed with BHT. The most marked changes have occurred in serum cholesterol and phospholipid levels. High dosage levels of BHT resulted in increased serum cholesterol and phospholipids in both male and female rats, although these levels appeared to return to normal after administration of antioxidants ceased (18,32,42). Pascal (43) found a 33% decrease in hepatic neutral fat when rats were fed diets containing 0.1-0.5% BHT, although Gaunt, et al., (32) reported that levels of liver cholesterol and phospholipid remained constant during treatment with 5 g BHT/kg/day for 14 days.

The mechanism of the effects of BHT upon lipid levels was studied by Johnson and Holdsworth (44), who injected rats with acetate-1-$^{14}$C. BHT given alone increased the rate of synthesis and turnover of body and liver fatty acids, but, when 0.5% BHT was fed with lard, the rate of synthesis of body and liver fatty acids was reduced. In both cases, cholesterol levels were increased. The authors postulated that BHT caused its effects by increasing the availability of acetyl-coenzyme A. According to Hathaway (39), other studies have indicated that changes in lipid levels result from alterations of phospholipid synthesis and transport of neutral fat in the liver.

When juvenile monkeys were dosed with 50 or 500 mg/kg body wt for 28 days, with the exception of cholesterol levels, few statistically significant alterations occurred in the lipid levels of the plasma and liver (40). In fact, BHA (500 mg/kg) prevented many lipid changes elicited by corn oil, which was used as a carrier for the antioxidants. BHA maintained higher levels of plasma lipid phosphorus and triglycerides and lower levels of liver cholesterol, liver lipid phosphorus, liver triglycerides, and plasma cholesterol (40). High doses of BHT had similar effects upon lipid levels but were not as effective as BHA. Also in contrast to effects in rodents, both 50 and 500 mg/kg/day of BHT caused significant decreases in plasma and liver cholesterol. High doses of BHA significantly decreased plasma and liver cholesterol; however, low doses significantly decreased only liver cholesterol.

It was the opinion of Branen, et al., (40) that BHA and BHT were acting in the monkey to spare vitamin E and allow it to perform its normal functions in lipid metabolism. There is much evidence to indicate that BHA, BHT, and other synthetic antioxidants spare vitamin E. Alfin-Slater (45) and Krisnamurthy and Bieri (46) reported that BHT and other synthetic antioxidants can prevent some of the symptoms of a vitamin E deficiency. BHT also was reported to act similarly to vitamin E in preventing dietary reticulocytosis (47) and encephalomalacia (48) in chickens; reducing the frequency of fetal resorptions in the rat (49);

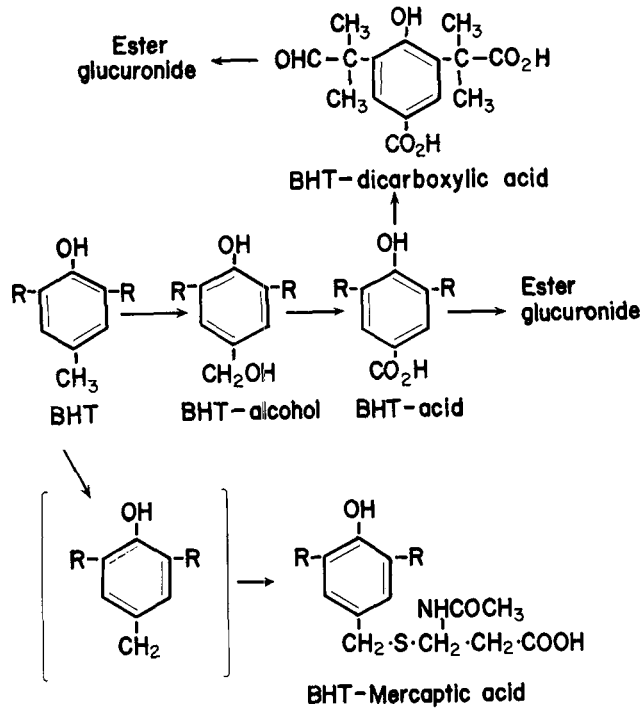


FIG. 3. Metabolic fate of butylated hydroxytoluene (BHT) (73). R represents tertiary-butyl group.

in preventing oxidation of rat perirenal fat (50), monkey liver fat (40), and pig and chicken fat (51); and in preventing fatty liver formation (52). Newberne, et al., (53) found that BHA and BHT decreased the effects of a choline deficiency on the liver, renal, and cardiovascular systems of rats, particularly by decreasing lesions in these tissues. Brown, et al., (11) found that BHT prevented atherosclerotic lesions upon extended feeding on a high fat diet; however, Wilson and Hartraft (54) found no effect of BHA or BHT upon prevention of experimental myocardial infarction in rats. These reported effects of BHT could explain the increase in life-span of mice fed 0.5% BHT on a semisynthetic diet (55,56).

Although the exact influence of BHA and BHT is not known, it appears reasonable to conclude that high doses of BHA and BHT influence lipid metabolism of primates and rodents. Further work is needed to clarify this role and to identify possible interrelationships between large doses of BHA and BHT, the level of dietary vitamin E, and the type and level of dietary lipid. Additional research is needed on the influence of synthetic antioxidants on prostaglandin synthesis which has been reported to be stimulated by low concentrations and inhibited by high concentrations of phenolic antioxidants (57). These effects on prostaglandins could be involved in the vascular response of certain individuals to BHA and BHT (58) and the reported inhibition of bradykinin action by BHA (59). To my knowledge, no reports exist on the influence of antioxidants on in vivo prostaglandin synthesis.

## EFFECTS OF BHA AND BHT UPON REPRODUCTION

The influence of BHA and BHT on the reproductive capacity of animals is still not clear. The earliest report of possible effects was by Brown, et al., (11) who reported that 3 of 30 litters from BHT fed rats had anopthalmic young. Clegg (60), however, attributed these effects to a deficiency of vitamin A in the diet used by Brown, et al. (11). In several other studies, no such effects were observed in rats or mice fed high levels of BHA or BHT (18,61,62). However, Johnson (61) reported some untoward effects on the reproductive capacity of mice, and Stokes, et al., (62)

also reported that 0.5% of BHA or BHT in the diet of female mice resulted in brain and behavioral changes in the newborn offspring, including decreased levels of seratonin and decreased activity of brain cholinesterase.

Allen and Engblom (21) recently completed a chronic study in which 6 adult female monkeys were given 50 mg BHA and 50 mg BHT/kg body wt/day for ca. 2 years. This level of antioxidants had no effect upon the general appearance, clinical chemistry, hematology, or reproductive capabilities of the adult females. The infants born of these females displayed no birth defects and were normal in all behavioral patterns.

## METABOLISM OF BHA AND BHT

Major differences have been reported to exist between rodents and primates in the pattern of metabolism, the rate of excretion, and causes of delayed excretion of BHA and BHT.

BHA was metabolized rapidly by all animal species studied. Within 24 hr, high quantities of BHA and its metabolites were excreted in the urine of rats (63,64), dogs (65), rabbits (66), monkeys (67), and man (68). Differences did exist, however, in the pattern of BHA metabolism of different animal species (Fig. 2). Rabbits excreted BHA primarily as glucuronide conjugates, although small amounts also were excreted as either sulfates and as the free phenols (66). In the rat, the predominante 3-tert-butyl isomer of BHA was converted primarily to the glucuronide ester, whereas the 2-tert-butyl isomer was converted mostly to ether sulfate (65). Dogs excreted BHA in the urine primarily as ether sulfates, although tert-butyl-hydroquinone also was formed (65). Man excreted 27-77% of a low dose of BHA as the glucuronide conjugate (65). No demethylated or hydroxylated products were found. It was concluded by Astill, et al., (65) that small doses of BHA in man and large doses in rats and rabbits have similar metabolic patterns.

In all animals species, BHT was excreted at a much lower rate than BHA, and major differences existed in the metabolism of BHT by rodents and primates. Golder, et al. (64), Ladomery, et al. (69), and Daniel and Gage (70) found that rats excreted 80-90% of an oral dose of radioactive BHT within 4 days, with 24-40% in the urine and the remainder in the feces. Rabbits excreted 54% of an oral dose in the urine (71). The apparent delay in the excretion of BHT by the rats has been attributed to enterohepatic circulation (72). Contrary to the excretion in rats, man and monkey excreted ca. 70-80% of a single oral dose in the urine (67,68). In monkeys, the remaining BHT was excreted in the feces (67). Daniel, et al., (68) concluded that there is no considerable enterohepatic circulation in man, and, because of this, BHT will accumulate to a greater extent in man than in the rat.

Differences also existed in the metabolic pattern of BHT by rodents and primates (Fig. 3). Daniel, et al., (72) showed that the major metabolites of BHT in the urine of rats were 3,5-di-t-butyl-4-hydroxy-benzoic acid (BHT-acid), both free and as a glucuronide, and S-(3,5-di-t-butyl-4-hydroxybenzyl)-N-acetylcysteine (BHT-mercaptic acid). BHT-acid was also the major urinary metabolite of rabbits (71). Ladomery, et al., (74) claimed that metabolites in the rat bile after parenteral injection were similar to those in the urine. They reported that BHT-acid was the major metabolite with minor amount of 3,5-di-t-butyl-4-hydroxybenzyl alcohol (BHT-alcohol), 3,5-di-t-butyl-4-hydroxy-benzaldehyde (BHT-aldehyde), and 1,2-bis-(3,5-di-t-butyl-4-hydroxyphenyl)ethane. Holder, et al., (75) concluded that BHT-acid was responsible for enterohepatic circulation of BHT in the rat and that the oxidation of BHT to BHT-acid controlled the elimination of BHT in the bile. BHT-acid was also the major metabolite in rat feces (73).

There is still some controversy regarding the metabolism of BHT by man. Daniel, et al., (73) reported that man excreted the bulk of BHT in the urine as a glucuronide of 4-carboxy-2-(1-carboxy-1-methylethyl)-6-(1-formyl-1-methylethyl) phenol or simply, BHT-dicarboxylic acid. Holder, et al. (76), however, did not find this metabolite and claimed that the major metabolites in man were BHT-acid and its ester glucuronide. Daniel, et al., (73) found only minor quantities of free and conjugated BHT-acid, and mercaptic acid derivatives were virtually absent. Daniel and Gage (77) claimed that the inability of Holder, et al., (76) to find BHT-dicarboxylic acid had resulted from analytical problems.

Monkeys which excrete BHT at a rate similar to that of man, excreted BHT primarily as a glucuronide of BHT-acid, and only minor quantities of other metabolites were found (67). More metabolic studies should be carried out on man to determine under what conditions BHT-dicarboxylic acid is formed.

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