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Effects of synthetic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ-like compounds*

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This study investigated the effects of the phenolic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ, MeIQ and MeIQx by the Ames test. Results demonstrated that MeIQ, IQ and MeIQx were all mutagenic when tested with the Ames test with their potency being in the order listed above. This was true on testing with both TA98 and TA100, both of which required added S-9 for activation. It was clearly demonstrated that BHA and PG significantly inhibited the mutagenicity of IQ, MeIQ and MeIQx. On the other hand, BHT had little effect on the mutagenicity of IQ and MeIQ at low concentrations, but significantly increased their mutagenicity at high concentrations. BHT slightly inhibited the mutagenicity of MeIQx at all concentrations tested.

ABBREVIATIONS

BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; PG = *n*-propyl gallate; IQ = 2-amino-3-methylimidazo-[4,5-f]quinoline; MeIQ = 2-amino-3,4-dimethylimidazo-[4,5-f]quinoline; and MeIQx = 2-amino-3,8-dimethylimidazo-[4,5-f]quinoxaline.

INTRODUCTION

IQ and MeIQ were originally isolated from broiled fish (Kasai *et al.*, 1980*a,b*) while MeIQx was isolated from beef (Kasai *et al.*, 1981). These compounds, which are classified as IQ-like compounds, were also present in beef extract and fried beef (Hayatsu *et al.*, 1983; Hargraves & Pariza, 1983; Turesky *et al.*, 1983; Felton *et al.*, 1984). All three of these IQ-like compounds are classified as mutagens by using the Ames *Salmonella typhimurium* test (Kasai *et al.*, 1980*a,b*, 1981; Hayatsu *et al.*, 1983; Felton *et al.*, 1984).

Many antioxidants have been shown to inhibit carcinogenesis (Ames, 1983). For example, BHA (Wattenberg, 1972; McKee & Tometsko, 1979), BHT (McKee & Tometsko, 1979) and PG (Rosin & Stich, 1978) all have been shown to reduce reversion induced by chemicals requiring metabolic activation in the Ames test. The present study was carried out to evaluate the effect of the phenolic antioxidants (BHA, BHT and PG) on the mutagenicity of IQ, MeIQ and MeIQx using the Ames test.

MATERIALS AND METHODS

Reagents

MeIQ and MeIQx were obtained as a gift from Dr. S. Grivas at the Swedish University of Agricultural Science, Lund, Sweden. IQ was purchased from Wako Chemicals USA, Inc. (Dallas, Texas). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *n*-propyl gallate (PG) were purchased from Sigma Chemical Company (St. Louis, Missouri).

Methods

IQ, MeIQ and MeIQx standards were carefully transferred and separately weighed into preweighed 2 gram vials to the nearest 0·0001 g. The weighed standards were dissolved quantitatively in dimethyl sulfoxide

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(DMSO). Through a series of dilution steps, each standard was diluted to give five different concentrations: 0·5, 5, 50, 500 and 5000 ng/μl. Each standard solution was then subjected to *Salmonella typhimurium* tester strains TA98 and TA100 to determine the most sensitive concentration range for each tester strain. Afterwards, five different concentrations in the most sensitive range of each standard were prepared and subjected to the Ames test (Maron & Ames, 1983). Standard curves for each compound tested for both tester strains were then prepared.

The effects of the antioxidants (BHA, BHT and PG) on the mutagenicity of IQ, MeIQ or MeIQx were evaluated in the present experiment. Based on the mutagenicity determined by previous experimentation, a concentration was chosen for each standard that was mutagenic to TA98 and TA100 + S-9. The concentrations used were 20 μg/plate for IQ, 3·5 μg/plate for MeIQ and 300 μg/plate for MeIQx.

In the Ames test, the S-9 fraction was prepared from Arochlor 1254-induced Sprague-Dawley rat liver. Both the S-9 fraction and the uninduced test standard were added to all culture tubes. Different concentrations of BHA, BHT and PG (0·1, 0·2, 0·3, 0·4, 0·5, 5, 10, 20, 30, 40 and 50 μg/plate) were tested as controls and were added to the test tubes containing the standards. The antioxidants (BHA, BHT and PG) were added to the tubes before addition of the tester strains. Each concentration was then tested in triplicate.

RESULTS

The concentration effects of IQ, MeIQ and MeIQx toward TA98 and TA100 with added S-9 were examined. All three compounds were toxic to TA98 in the presence of S-9 at all levels above 100 μg/plate. Concentrations in the range 0 to 0·5 μg/plate were tested and the results are presented in Fig. 1. In this range the specific activities of MeIQ, IQ and MeIQx were 5644, 3836 and 652 revertants/μg, respectively. Thus, MeIQ showed the most mutagenicity and was followed by IQ and MeIQx in that order. These results are in agreement with the findings of Sugimura (1982, 1986) and of Felton (1987), who have demonstrated that MeIQ is the most potent mutagen and is followed in order by IQ and MeIQx.

On testing with TA100 + S-9, MeIQ was more toxic than IQ and MeIQx. The mutagenic test results for these compounds in the most sensitive concentration range are presented in Fig. 2. In this range, the specific activities of MeIQ, IQ and MeIQx were 540, 261 and 154 revertants/μg, respectively. The mutagenicity of MeIQ when tested with TA100 + S-9 was higher than that for IQ and MeIQx as shown in Fig. 2. Results of this study demonstrated that the MeIQ, IQ and MeIQx were all mutagenic when tested with the Ames test with their potency being in the order listed above. This was true on testing with both TA98 and TA100, both of which required added S-9 for activation.

To determine the effects of antioxidants on their mutagenicity, the concentrations of each of these IQ-like compounds tested in this study were taken from the midpoint of the linear portion of the concentration response curve. The concentrations chosen were 20 μg/plate for IQ, 3·5 μg/plate for MeIQ and 300 μg/plate for MeIQx. Different concentrations of each of the antioxidants (BHA, BHT and PG) were added to each of the mutagens (IQ, MeIQ and MeIQx) in order to determine their effects upon the mutagenic response.

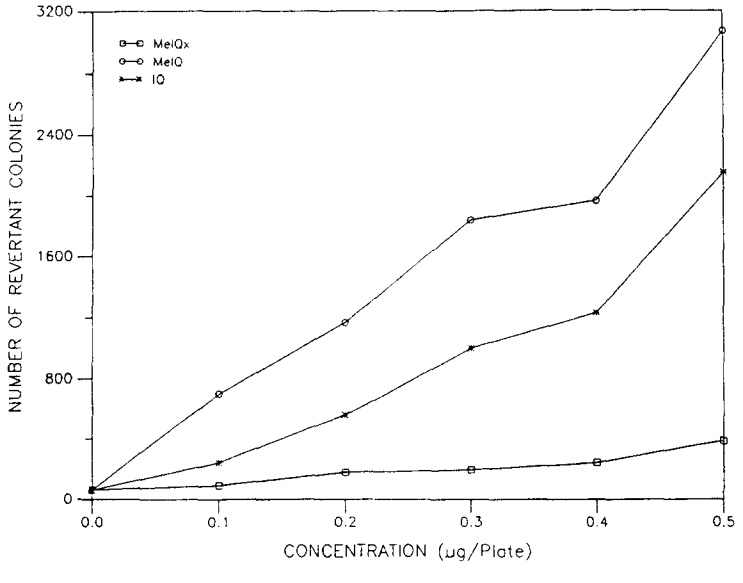

Fig. 1. Concentration effects of IQ, MeIQ and MeIQx with TA98 + S-9. Spontaneous revertants have been subtracted. Concentration range was from 0 to 0·5 μg/plate.

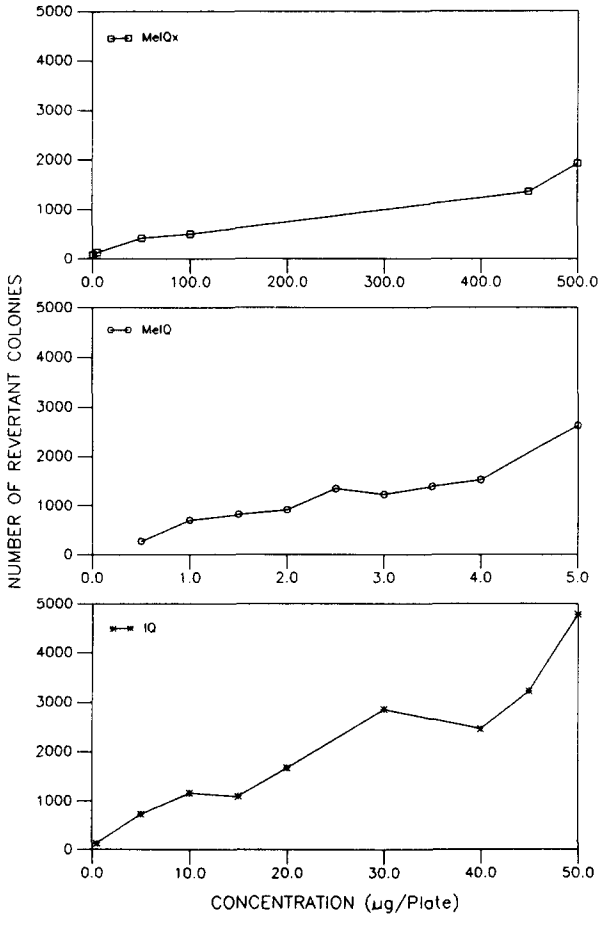

Fig. 2. Concentration effects of IQ, MeIQ and MeIQx with TA100 + S-9. Spontaneous revertants have been subtracted.

In the present study, the antioxidants tested (BHA, BHT and PG) were not mutagenic to TA98 and TA100 at all concentrations tested either with or without the S-9 fraction. On testing IQ with BHA, BHT or PG at concentrations ranging from 0 to 5000 μg/plate with TA100 + S-9, it was demonstrated that BHA and PG are both concentration-responsive and inhibit mutagenicity. When the amount of BHA and PG added was below 500 μg/plate, these two antioxidants exhibited inhibitory effects on the mutagenicity of IQ, which were not due to toxicity as demonstrated by the presence of the background lawn. However, upon adding 500 μg/plate of either BHA or PG to the test solution (IQ + antioxidant + tester strain + S-9), the solutions became cloudy and interfered with identification of the background lawn. This made it difficult to determine if the decrease in growth was due to an antimutagenic effect or if it was associated with toxicity of the antioxidants. Therefore, the three antioxidants were added at concentrations of 0 to 50 μg/plate, and the test was repeated with the results being shown in Fig. 3. The inhibitory effects of BHA and PG towards the mutagenicity of IQ were confirmed by testing against TA100 + S-9. Results demonstrated that the two antioxidants had an antimutagenic effect against IQ. On the other hand, BHT had little effect on the mutagenicity of IQ on testing with TA100 + S-9 in both trials.

When tested with TA98 + S-9 (Fig. 4), both BHA and PG inhibited the mutagenicity of IQ, which was shown to not be the result of any toxic effects for either additive. On the other hand, BHT significantly increased the mutagenicity of IQ (Fig. 4). On testing MeIQ with TA100 + S-9, BHA and PG had similar inhibitory effects as observed on testing with IQ (Fig. 5). BHT decreased the mutagenicity of MeIQ at a dose of 5 μg/plate. On testing concentrations from 50 to 5000 μg/plate, however, BHT significantly increased the mutagenicity of

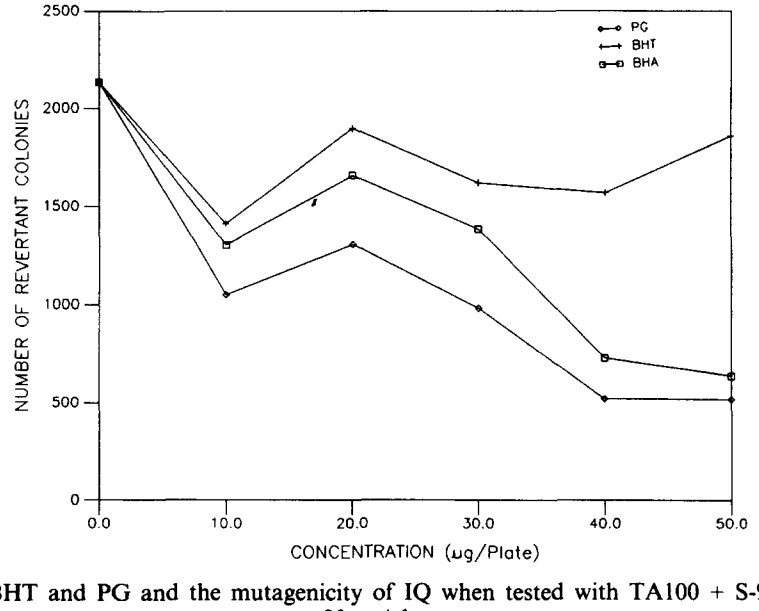

Fig. 3. Effects of BHA, BHT and PG and the mutagenicity of IQ when tested with TA100 + S-9 at an IQ concentration of 20 μg/plate.

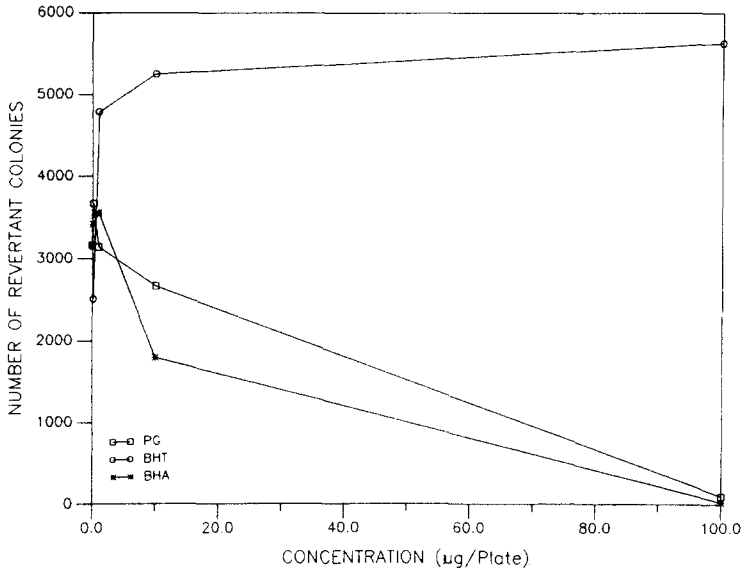

Fig. 4. Effects of BHA, BHT and PG on the mutagenicity of IQ when tested with TA98 + S-9 at an IQ concentration of 20 μg/plate.

MeIQ. To confirm the results, concentrations ranging from 0 to 50 μg/plate for BHA, BHT and PG were repeated (Fig. 6). BHA and PG decreased the mutagenicity of MeIQ at all concentrations tested. On the other hand, BHT had little effect on the mutagenicity of MeIQ toward TA100 + S-9 at concentrations below 50 μg/plate. At higher concentrations (above 50 μg/plate), however, BHT increased the mutagenicity of MeIQ (Fig. 5).

When tested with TA98 + S-9 (Fig. 7), a similar pattern was observed. BHA and PG were concentration-responsive and inhibited the mutagenicity of MeIQ. On the other hand, BHT slightly decreased the mutagenicity of MeIQ at a concentration of 10 μg/plate, but at all higher concentrations it increased the mutagenicity of MeIQ in a concentration-responsive manner (Fig. 7).

On testing MeIQx with TA100 + S-9 (Fig. 8), both BHA and PG inhibited the mutagenicity of MeIQx in a concentration-responsive manner. BHT, however, had a minor inhibitory effect on MeIQx, but much less than was the case for BHA and PG (Fig. 8).

DISCUSSION

The values for the mutagenic activity of IQ, MeIQ and MeIQx detected in this study are somewhat lower than those reported previously (Nagao *et al.*, 1981; Sugimura,

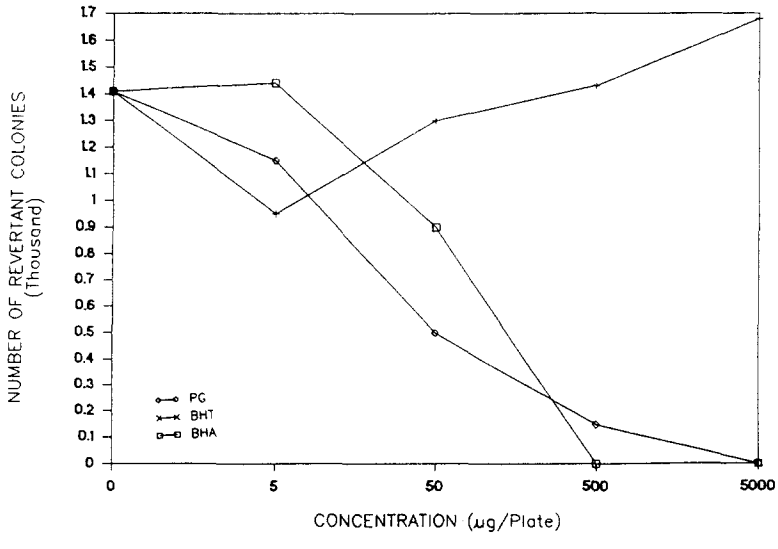

Fig. 5. Effects of BHA, BHT and PG at 0–5 μg/plate on the mutagenicity of MeIQ when tested with TA100 + S-9 at an MeIQ concentration of 3·5 μg/plate.

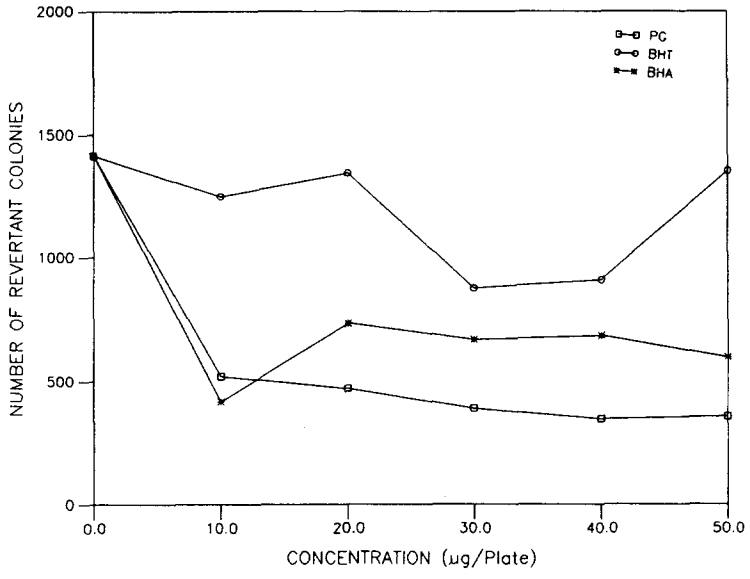

Fig. 6. Effects of BHA, BHT and PG at 0–50 µg/plate on the mutagenicity of MeIQ when tested with TA100 + S-9 at an MeIQ concentration of 3·5 µg/plate.

1986). The differences can likely be explained by the way that the Ames test was carried out in the present study, with the S-9 fraction or phosphate buffer being added first, followed by addition of the test solution, and then by the properly diluted bacterial culture. The tubes were gently mixed and incubated at 37°C for 20 min. After incubation, the molten top agar at 45°C was added to the mixture, vortexed and poured onto a minimum glucose-agar plate that was tilted and rotated to achieve uniform distribution. The plates were counted and compared with controls after 48 h at 37°C. In spite of the somewhat lower values of IQ, MeIQ and MeIQx, the values seem reasonable in comparison to control samples and demonstrate that all three compounds were mutagenic.

Probably the hardest finding to verify in comparison to other work is the mutagenicity result for BHT, which indicated that BHT increased the mutagenicity of IQ and MeIQ at high concentrations. On the other hand, BHA inhibited mutagenicity at all concentrations tested. It is of interest to note that BHA has been reported to cause proliferative and neoplastic lesions of the epithelium in the forestomach of rats (Masui *et al.*, 1986) and hamsters (Ito *et al.*, 1983). although BHA shows no evidence of mutagenicity with the Ames *Salmonella typhimurium* test using tester strains TA97,

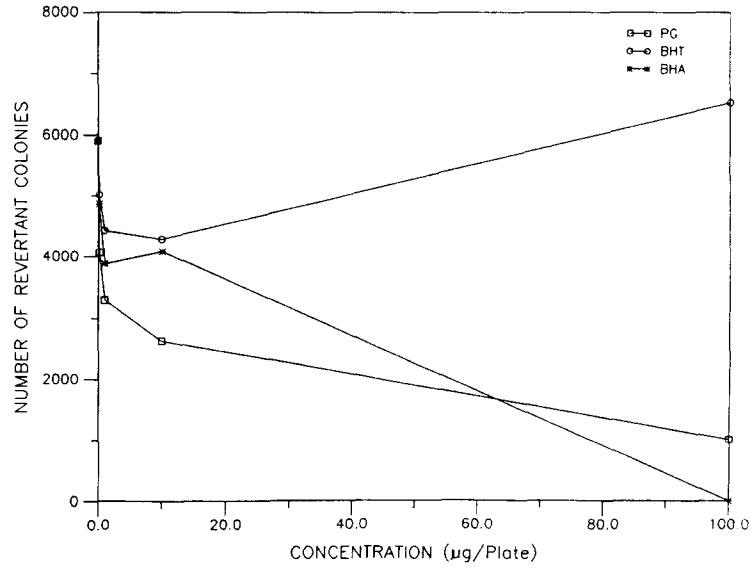

Fig. 7. Effects of BHA, BHT and PG on the mutagenicity of MeIQ when tested with TA98 + S-9 at an MeIQ concentration of 3·5 µg/plate.

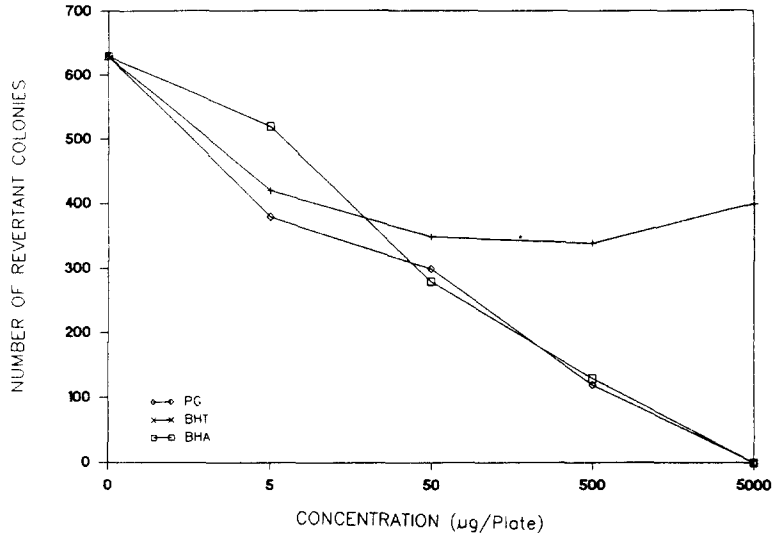

Fig. 8. Effects of BHA, BHT and PG on the mutagenicity of MeIQx when tested with TA100 + S-9 at an MeIQx concentration of 300 µg/plate.

TA98, TA100, TA104, TA1535, TA1537 and TA1538 (Joner, 1977; Batzinger *et al.*, 1978; Rosin & Stich, 1978; Bonin & Baker, 1980). Ito & Hirose (1989) have reviewed the evidence for the carcinogenic and chemopreventive properties of antioxidants and concluded that, even though BHA induced tumorigenicity in the forestomach of rats and hamsters, this finding is probably of little or no significance to human cancer since humans do not have a forestomach. Nevertheless, Phillips *et al.* (1989) have suggested that extracellular generation of reactive oxygen species may be involved in BHA-induced clastogenicity *in vitro*. This indicates the need for further research on the possible role of the synthetic antioxidants (BHA and BHT) on mutagenicity and carcinogenesis, especially as related to cancer in the forestomach of rodents.

BHT consumption has been reported to inhibit carcinogenesis by McKee & Tometsko (1979) as well as by Wattenberg (1972) and by Ito & Hirose (1989). In rodent studies, however, there is evidence that BHT causes hepatic cancer (Lindenschmidt *et al.*, 1986; Olson *et al.*, 1986; Inai *et al.*, 1988). BHT has also been demonstrated to enhance chemically induced mutagenesis and carcinogenesis (IARC, 1986; Ito & Hirose, 1989). In the present study, high concentrations of BHT were shown to enhance the mutagenic effects of IQ and MeIQ which provides further evidence that BHT can promote the mutagenicity of certain chemicals. However, BHT only increased the mutagenic effects of IQ and MeIQ at high concentrations. Thus, the increase in mutagenesis caused by BHT does not necessarily involve any increase in human cancer risk. Nevertheless, further studies on the roles of both BHA and BHT in cancer of humans and other animals would seem to be warranted.

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