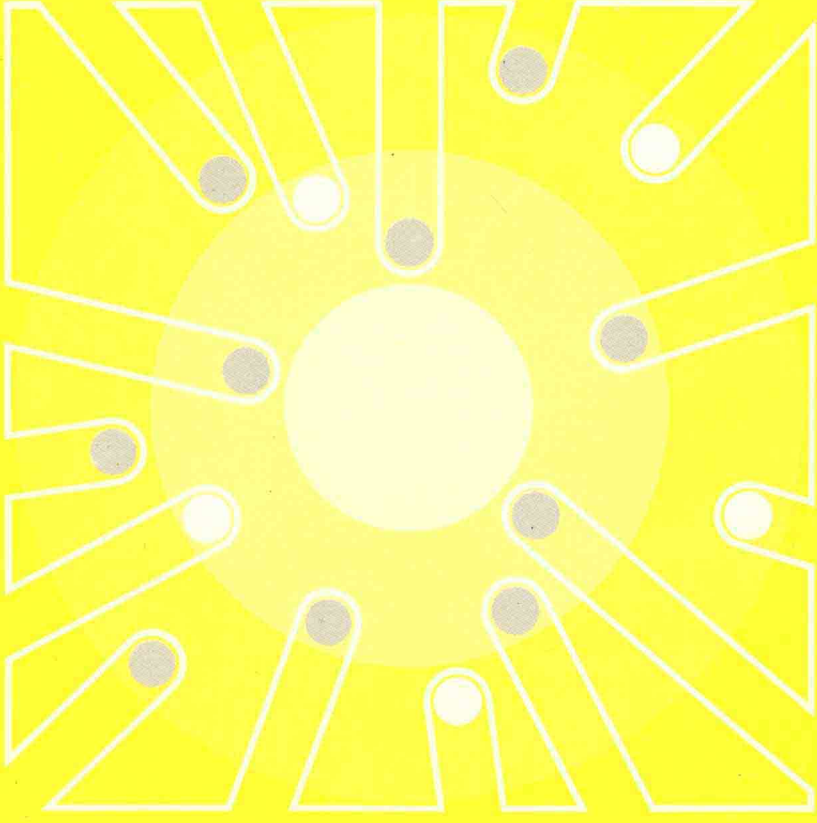
WHO FOOD ADDITIVES SERIES 40

Safety evaluation of certain food additives and contaminants

Prepared by
THE FORTY-NINTH MEETING OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA)



IPCS

International Programme on Chemical Safety
World Health Organization, Geneva

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IPCS - International Programme on Chemical Safety

The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer-review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Health & Environment International, Ltd., by agreement with WHO, performed independent literature searches on some of the substances on which data are summarized in this document in order to ensure that all relevant toxicological and related information was reviewed.

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PREFACE

The monographs contained in this volume were prepared at the forty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Rome, Italy, 17-26 June 1997. These monographs summarize the safety data on selected food additives and contaminants reviewed by the Committee. The data reviewed in these monographs form the basis for acceptable daily intakes (ADIs) established by the Committee.

The forty-ninth report of JECFA will be published by the World Health Organization in the WHO Technical Report Series. Terms abbreviated in the monographs are listed in Annex 2. The participants in the meeting are listed in Annex 3 of the present publication and a summary of the conclusions of the Committee is included as Annex 4. Some of the substances listed in this annex were evaluated at the present meeting for residues only.

Specifications established at the forty-ninth meeting of JECFA have been issued separately by FAO as Food and Nutrition Paper No. 52, Add. 5. These toxicological monographs should be read in conjunction with the specifications and the report.

Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States, and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives and Contaminants and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants, and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The toxicological monographs contained in this volume are based upon working papers that were prepared by Temporary Advisers. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers.

Many proprietary unpublished reports are cited. These were voluntarily submitted to the Committee by various producers of the food additives under review and in many cases these reports represent the only safety data available on these substances. The Temporary Advisers based the working papers they developed on all the data that were submitted, and all these studies were available to the Committee when it made its evaluations. Special

acknowledgement is made to these advisers. The monographs were edited by Dr P.G. Jenkins, International Programme on Chemical Safety.

From 1972 to 1975 the toxicological monographs prepared by JECFA were published in the WHO Food Additives Series; after 1975 this series was available in the form of unpublished WHO documents provided upon request to the Organization. WHO Food Additives Series No. 20, which was prepared by the twenty-ninth Committee in 1985, through to WHO Food Additives Series No. 24, which was prepared by the thirty-third Committee in 1988, were published by the Cambridge University Press. Beginning with WHO Food Additives Series No. 25, which was prepared by the thirty-fourth Committee, WHO has been producing these volumes as priced documents.

The preparation and editing of the monographs included in this volume have been made possible through the technical and financial contributions of the Participating Institutions of the IPCS, which supports the activities of JECFA. IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization, which is the executing agency. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

Any comments or new information on the biological or toxicological data on the compounds reported in this document should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Expert Committee on Food Additives, International Programme on Chemical Safety, World Health Organization, Avenue Appia, 1211 Geneva 27, Switzerland.

CONTENTS

ANTIOXIDANT

tert-BUTYLHYDROQUINONE (TBHQ)

First draft prepared by
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1. EXPLANATION

tert-Butylhydroquinone (TBHQ) was evaluated by the Committee at its nineteenth, twenty-first, thirtieth, thirty-seventh and forty-fourth meetings (Annex 1, references 38, 44, 73 94 and 116). At the forty-fourth meeting, the previously-

established temporary ADI of 0-0.2 mg/kg bw was extended pending results from ongoing long-term studies in rodents. This ADI was derived from a NOEL of 1500 mg/kg of feed (equivalent to 37.5 mg/kg bw per day) in a 117-week feeding study in dogs on the basis of haematological changes observed at the next highest dose level of 5000 mg/kg feed (Annex 1, reference 39).

At its present meeting, the Committee reviewed the results of the long-term studies in mice and rats. In addition, new information relating to metabolism of TBHQ, its effects on enzyme induction and its short-term and reproductive toxicity in rodents was available for review. The results from the long-term study in dogs and the genotoxicity studies relating to clastogenic potential of TBHQ were also re-evaluated.

The following consolidated monograph is a compilation of studies from the previous monographs and those reviewed for the first time at the present meeting.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution, excretion

2.1.1.1 Rats

In a single dose study, rats received ^{14}C-labelled TBHQ equivalent to 15, 48, 92, 383, 380 or 400 mg/kg bw. Urine and faeces were collected daily as was expired CO_2. At the end of the test period the animals were sacrificed and blood, brain, kidneys, liver, gastrointestinal tract, and perirenal, omental and subcutaneous fat removed for assay. Of the administered radioactivity, 78-88% was recovered in the urine, the bulk of this being excreted within the first 24 hours (55-82.7% of the administered dose). Of the recovered radioactivity, 70-76% was in the form of the *o*-sulfate conjugate and 1-2% as the *o*-glucuronide. Faecal excretion was 2-6%. Only traces of radioactivity were detected in the tissues at the 92 mg/kg bw level; no values were given for the higher levels (Astill *et al.*, 1967a).

In another experiment, rats (body weight 200-250 g) were maintained on a daily diet that allowed an intake of 5.7 mg/kg bw (0.029% level) of ^{14}C-TBHQ daily for 17 days. Urine and faeces were collected throughout the experiment. At the end of the test period the rats were starved overnight before sacrifice, and brain, liver, kidney, and fat samples collected. Tissue levels were as follows (mg TBHQ/g wet tissue): liver, 0.06-0.34; kidney, 0.09-0.38; brain, 0.06-0.56; fat, 0.06-0.37 (Astill *et al.*, 1967a).

Male and female rats (body weight 250 g) were given a single dose of TBHQ, dissolved in corn oil (10% w/w), by intubation at dose levels equivalent to 100, 200, 300 or 400 mg/kg bw. At the 400 mg/kg bw level there was a rapid onset

of ataxia, followed by recovery in 2-3 hours. Urine samples were collected daily for 3 days before dosing and then for 6 days after dosing. At all dose levels excretion appeared to be complete in 3-4 days. About 66% of the dose was excreted as the *o*-sulfate conjugate and less than 10% as the glucuronide. At the 100 mg/kg bw level, urinary excretion accounted for almost all the dose. At higher levels about 33% could not be accounted for in the urine or be detected in the faeces. Excretion of the free TBHQ at the 100 mg/kg bw level was about 12%, but this decreased at the higher dose levels (2% at 400 mg/kg bw). No other major metabolites were detected (Astill *et al.*, 1968).

Urine samples were collected from two animals of each of the 0, 0.16 and 0.5% dietary TBHQ groups of a long-term feeding study at months 12 and 20. Serum samples were collected from groups of five rats at months 6, 12, 20 and at autopsy. Samples of perirenal, omental and subcutaneous fat were removed at autopsy, and pooled by sex and dose. At 12 months, males at both levels excreted about equal amounts of the conjugates in the urine (*o*-sulfate and *o*-glucuronide). Most (about two-thirds) of the excretory products in females was the *o*-sulfate form and the remainder the *o*-glucuronide. At 20 months in both male and female, most of the conjugate excreted was in the *o*-sulfate form with little evidence for glucuronide excretion. Only negligible amounts of TBHQ were detected in serum or fat (Astill *et al.*, 1968).

Portions of the fat of control animals and animals that had been maintained on 0.5% TBHQ were examined for stability by the active oxygen method (oxidative stability) and also for TBHQ content. There were no apparent differences in the oxidative stability of fats from treated and control animals, nor did polarographic and colorimetric methods of analysis (sensitive to 5 mg/kg) indicate the presence of TBHQ in the fat of test animals (Eastman Chemical Products, 1968a).

Pregnant albino SD rats (380-440 g body weight; age 48 weeks) were selected from the third litter of second generation females in a reproductive toxicity study that had received 0.5% of TBHQ in the diet since weaning. Animals were given one day before term an oral dose of ^{14}C-TBHQ (40 mg/kg bw) as a 10% solution in corn oil. Urine and faeces were collected up to time of sacrifice (7.6-16.7 hours after dosing). Fetuses were removed by Caesarean section. The uterus, amniotic fluid, gastrointestinal tract, liver, brain, kidneys and fat specimens were collected for radioassay. About 74% of the dose was excreted in the urine in the 16.7-hour period. Only 10% of the dose was detected in the gastrointestinal tract at 7.4 hours after dosing, and 8.5% at 17.6 hours. The level of radioactivity in fetuses was 0.2% of the dose at 7.6 hours and 0.02% at 16.7 hours. Similar small proportions of the dose were present in the uterus and amniotic fluid and other tissues examined. Based on these results, extrapolation to possible known exposures suggest that at the highest possible intake (0.1 mg/kg bw per day), the human fetus would be exposed to the order of 1% of the daily intake in the form

of unchanged TBHQ and probably higher levels of the conjugate (Astill & Walton, 1968).

Oral doses of 4 ml of 0.01, 0.1 or 1.0% butylated hydroxyanisole (BHA) were administered to male F344 rats. After 3 hours, the concentrations of *tert*-butylquinone (TBQ) (the oxidation product of TBHQ) detected by HPLC in the forestomach mucosa were found to be 0.00453, 0.04504 and 0.05520 µg/animal, respectively, compared with 1.77, 18.84 and 216.28 µg BHA/animal, respectively (Morimoto *et al.*, 1991).

TBHQ was not detected in homogenates of forestomach mucosa from male F344 rats that had received 4 ml of 0.01-2.0% ^{14}C-BHA. Forestomach homogenates were therefore treated with sodium dodecyl sulfate in order to reduce TBQ to TBHQ, in which form it could be more easily measured. The TBHQ content thus generated in the forestomach homogenates was proportional to the dose of BHA. The ratios of the total tissue content of TBHQ to the total amount of covalent binding of ^{14}C in forestomach were 0.01-0.03% at oral BHA doses of 0.1-2.0%. The authors concluded that the covalent binding level was an important indicator of reactive metabolites of BHA (Morimoto *et al.*, 1992).

2.1.1.2 Dogs

Male beagle dogs (about 11 kg weight) were fed Purina Chow and TBHQ as a single 100 mg/kg bw oral dose via ground meat capsule. Urine was collected 3 days before dosing and 6 days after dosing. Excretion was essentially complete within 48 hours. The major urinary excretory products were the *o*-sulfate, and *o*-glucuronide conjugates and a small amount of unchanged TBHQ. Total recoveries ranged from 77 to 98%. Most (about two-thirds) of this was as the *o*-sulfate and one-third as the *o*-glucuronide (Astill *et al.*, 1967b).

In another study, 26 male and female dogs were used. The dogs were maintained on diets containing TBHQ dissolved in corn oil at levels equivalent to 0, 0.05, 0.1 or 0.5%. Urine and serum samples were collected on day 9 and one day before commencement of feeding TBHQ, and at months 3, 6, 12, 13 and 24 of the test period. Serum was collected 23 hours after feeding. At autopsy, performed on one dog of each sex at each dose level at 12 months, and on the remaining dogs at 24 months, samples of perirenal, omental and subcutaneous fat were removed. Chromatographic studies of the urine indicated excretion of both the *o*-sulfate and *o*-glucuronide conjugates, at all dose levels. In the case of males, the *o*-sulfate/glucuronide ratios were 2/1, whereas in females the bulk of the conjugate was in the form of the *o*-sulfate. Only insignificant quantities of TBHQ were detected in the fat (the maximum in males was 7 mg/kg and in females 17 mg/kg, but in most cases the value was 0), and serum (0-0.7 mg/litre) (Astill *et al.*, 1967b).

Portions of the fat from test animals and animals that had been maintained on the highest level of TBHQ (0.5%) for 2 years were examined for stability by the active oxygen method. There was no apparent difference in the oxidative stability of fats from treated or control animals (Eastman Chemical Products, 1968a). In another study TBHQ residues were assayed in fat, brain, liver and kidney of dog and rats from the long-term feeding studies. Storage appeared to be negligible (Astill & Jones, 1969).

2.1.1.3 Humans

Human subjects (males) received TBHQ under the following conditions: (1) a gelatin capsule containing 150 mg TBHQ; (2) a mixture of TBHQ (2%) in corn oil and graham cracker crumbs, equivalent to a dose of 125 mg TBHQ; (3) 100 mg dissolved in cottonseed oil contained in a gelatin capsule; (4) 20 g of mixture containing TBHQ, 2% cottonseed oil and 2% confectioners' sugar in graham cracker crumbs. Doses of TBHQ ranged from 20 to 70 mg. Subjects one, two and three drank milk immediately after ingesting test material; subject four ate doughnuts and drank coffee.

Urine was collected from subjects 24 hours before dosing and during the 72-hour period after dosing. Blood was collected by veni-puncture at 3 or 5 and 24 hour after-dosing. Clinical observations were made immediately before ingestion and 3 to 6 hours after, and consisted of blood pressure, pulse response, condition of pharynx, conjunctivae and pupils and neurological effects. Haematological studies consisted of haemoglobin, cell volume, WBC, differentials, reticulocyte and platelet counts, and total protein. Urinalysis consisted of SpGr, albumin, reducing sugars, ketone bodies, occult blood, pH and sediment. Levels of TBHQ in serum and metabolites of TBHQ in urine were also determined.

There was no evidence of any systemic effect following ingestion of TBHQ. No significant changes were observed in haematological studies or urinalysis. Examination of urine indicated that TBHQ was excreted as the *o*-sulfate and *o*-glucuronide conjugates (ratio approximately 3:1). These were mainly recovered during the first 24 hours. No free TBHQ was detected at any time. The manner of ingestion had a marked effect on the proportion of the dose recovered from urine. TBHQ administered by methods 1 and 3 resulted in only 22-4% of the dose being recovered in the urine, whereas method 2 resulted in 90-100% recovery. In all cases, the same metabolic products were present in urine. High recoveries of TBHQ metabolites in urine were accompanied by a serum level of 31-37 mg TBHQ/litre at 3 hours for subject two, compared to 4-12 mg/litre for subjects one and three. At 24 hours these levels had fallen to 15 mg/litre for subject two and 2-12 mg/litre for subjects one and three (Astill *et al.*, 1967c).

2.1.2 Biotransformation

Following the intraperitoneal administration of 400 mg BHA/kg bw or 200 mg TBHQ/kg bw TBHQ to male Wistar rats, two previously undocumented

metabolites, 3-*tert*-butyl-5-methylthiohydroquinone (TBHQ-5-SMe) and 3-*tert*-butyl-6-methylthiohydroquinone (TBHQ-6-SMe), were detected in the urine using GC-MS. The authors suggested that these metabolites resulted from the metabolic conversion of glutathione conjugates of a quinone or semiquinone form of TBHQ. In rat liver microsomal preparations, the formation of two GSH conjugates at the 5- and 6- positions of TBHQ in the presence of an NADPH-generating system, molecular oxygen and GSH was confirmed. It appeared that glutathione S-transferase (GST) is not required for the reaction. While inhibitors of cytochrome P-450 markedly reduced formation of TBHQ-GSH conjugates, indicating its role in the activation of TBHQ to *tert*-butylquinone (TBQ) autooxidation was also shown to play a partial role in this reaction (Tajima *et al.*, 1991).

Benzylthiol derivatives synthesized from TBQ had higher first reduction potentials than the parent compound. The authors concluded that TBQ maintained its potential for the generation of active oxygen species even after its addition to cellular thiols (Morimoto *et al.*, 1991).

The *tert*-butyl semiquinone radical was shown to be formed from TBHQ in aerobic rat liver microsomes in the presence of NADPH. A concentration of 500 μM TBHQ and 5 μM TBQ produced similar reductions in SOD-inhibitable cytochrome c, which was used as an indication of excess superoxide anion radical production. The authors concluded that autooxidation of the semiquinone formed from the quinone was responsible for superoxide formation and that the hydroquinone entered the redox cycle via autooxidation. TBQ, but not TBHQ, induced toxic injury to rat hepatocyte plasma membrane as indicated by LDH release into the culture medium. The authors speculated that semiquinone-dependent superoxide formation was responsible for the toxic action (Bergmann *et al.*, 1992).

Incubation of TBHQ with horseradish peroxidase and hydrogen peroxide resulted in its rapid oxidation to TBQ. TBQ epoxide was also produced at hydrogen peroxide concentrations of 2.5 mM or more. The presence of horseradish peroxidase was not a requirement for the production of TBQ epoxide from TBQ (Tajima *et al.*, 1992).

Three GSH conjugates were generated by the incubation of TBHQ with GSH; two of these were monoconjugates at the 5 or 6 positions (*tert*-butyl group at position 2) and one was a 5,6 diconjugate. The redox potentials for the conjugates were twice those for the unconjugated hydroquinone. The mono-conjugates showed an approximately 10-fold increase in redox cycling activity (oxygen consumption in the presence of a reducing agent) compared with TBHQ, whereas the diconjugate showed a 2-fold increase compared with TBHQ. None of the major GST isoenzymes were required for the formation of glutathione conjugates from TBHQ (van Ommen *et al.*, 1992).

Incubation of TBHQ in phosphate-buffered saline resulted in the generation of the semiquinone radical through autooxidation, accompanied by the formation of superoxide anion, hydroxyl radical and hydrogen peroxide as detected by electron spin resonance (ESR) spectroscopy. The addition of prostaglandin H synthase resulted in a substantial increase of semiquinone production with concomitant production of reactive species. Under the conditions of the assay, lipoxygenase had no effect on the formation of the semiquinone. The presence of either prostaglandin H synthase or lipoxygenase was found to accelerate substantially the metabolism of TBHQ to TBQ compared with the rates of autooxidation. In an *in vivo* study, male Wistar rats were fed diets containing 1.5% BHA for 14 days, with concurrent administration of the prostaglandin H synthase inhibitors acetylsalicylic acid (0.2%) or indomethacin (0.002%) in the drinking-water. Both agents produced a significant decrease in the amount of TBQ excreted into the urine, compared with controls receiving drinking-water only, while the combined urinary excretion of BHA and its metabolites, TBHQ and TBQ, was similar for the various groups (46.9%, 45.4% and 43.5% of the ingested dose during urine collection in the control, indomethacin and acetylsalicylic acid groups, respectively). The results suggested an *in vivo* role for prostaglandin H synthase in the metabolism of TBHQ to TBQ (Schilderman *et al.*, 1993a).

Following intraperitoneal administration of TBHQ (1.0 mmol/kg bw) three glutathione metabolites, 2-*tert*-butyl-5-glutathione-*S*-ylhydroquinone, 2-*tert*-butyl-6-glutathione-*S*-ylhydroquinone and 2-*tert*-butyl-3,6-bisglutathion-*S*-ylhydroquinone, were identified in the bile of male F344 rats. Sulfur-containing metabolites of TBHQ were identified in the urine. The results indicated that TBHQ undergoes oxidation and GSH conjugation *in vivo* in the male F344 rat. These conjugates are excreted into the bile and undergo further metabolism prior to excretion in the urine. The authors suggested that the sulfur-containing metabolites of TBHQ may occur in amounts sufficient of play a role in the toxicity of TBHQ for kidney and bladder (Peters *et al.*, 1996a).

2.1.3 Effects on enzymes and other biochemical parameters

Adult male rats (SD strain) were maintained on standard diets containing the following additions: (1) none; (2) 5% heated cottonseed oil; (3) DL-ethionine, 2.5% level for 10 days; (4) 100 mg/kg bw per day phenobarbital for five days (intraperitoneal injection), (5) 1% corn oil + 0.05% BHA; (6) 4% corn oil + 0.2% BHA; (7) 1% corn oil + 0.05% TBHQ; (8) 4% corn oil + 0.2% TBHQ; and (9) 5% heated cotton-seed oil + 0.025% TBHQ. A liver microsomal fraction was prepared from each group of animals and glucose-6-phosphatase (G-6-Pase), *p*-nitroanisole demethylase (*p*NaD) and aniline hydroxylase (AHase) activities determined. The expected elevation of *p*NaD (5x) and AHase (3x) occurred with phenobarbital, but DL-ethionine had no significant effect on these enzymes. Phenobarbital produced a depression of G-6-Pase activity (25%), and TBHQ at 0.05% level produced a 25% depression in G-6-Pase which was absent at the 0.2% level. TBHQ had no

effect on *p*NaD at the 0.05% level, but produced at the 0.2% level a 60% elevation of *p*NaD. There was no clear effect on AHase. In contrast, BHA produced a 30% decrease in G-6-P at both levels, a 50% increase in *p*NaD at 0.05% and a 700% increase of *p*NaD at the 0.2% level. There was no effect on AHase. Inclusion of heated oil in the diet had no marked effect on previous changes. In another experiment in which measurements were made of enzyme activities in microsomal preparation from livers of rats fed for 180 days diets containing 0.5% TBHQ dissolved in either heated or unheated cottonseed oil, no significant differences were observed that could be attributed to heat treatment of oil before addition to the diet (Tischer & Walton, 1968).

*p*NaD, AHase, and G-6-Pase activities of microsomal fractions from dogs that had been maintained on diets containing 0, 0.05, 0.16 and 0.5% TBHQ for 2 years were within the range of control values (Tischer & Walton, 1968).

Electron microscopy studies of liver and kidney tissue from both dog and rat showed that long-term administration of TBHQ did not significantly alter the subcellular constituents or cause a proliferation of the endoplasmic reticulum of liver cells (Wolf & Fassett, 1968a,b).

The effects of antioxidants, including TBHQ, on prostaglandin biosynthesis were examined by determining the production of prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) by incubated microsomal fractions of bovine seminal vesicles. All the antioxidants tested proved to be concentration-dependent inhibitors of prostaglandin biosynthesis. A 50% inhibition of PGE_1 and PGE_2 biosynthesis was observed at TBHQ concentrations of 5.5 and 6.1 μM, respectively. A 50% inhibition was also observed with BHA at comparable concentrations, while much higher concentrations of butylated hydroxytoluene (BHT) and ascorbate were required to have the same inhibitory effect (Boehme & Branen, 1977).

Six phenols, including TBHQ and BHA, were examined for their ability to induce hepatic mono-oxygenase and detoxication enzyme activities in female CD-1 mice. TBHQ treatment (42 nmol/kg diet for 12 days) had no effect on relative liver weight, cytochrome P-450 content or on the activities of AHase, aminopyrine N-demethylase or peroxidase. TBHQ treatment was shown to increase cytosolic GST activity 2-fold while UDP-glucuronyl transferase (UGT) activity was reduced by one third. In an additional *in vitro* experiment with mouse liver microsomes, TBHQ was shown to inhibit benzo(*a*)pyrene (BP) metabolism and its DNA-binding capacity. These effects were not observed in the *in vivo* study (Rahimtula *et al.*, 1982).

Groups of 50 rainbow trout were fed diets containing 0 or 5.6 mmol/kg TBHQ (equal to 0.1%), BHT, BHA or ethoxyquin for 6 weeks. The treated trout had reduced liver/body weight ratios. Compared to controls, TBHQ treatment led to a decrease in hepatic microsomal protein and cytochrome P-450 content, and