



BUTYLATED HYDROXYTOLUENE (BHT)

<http://www.inchem.org/documents/jecfa/jecmono/v18je06.htm>

Explanation

This substance has been evaluated for acceptable daily intake for man (ADI) by the Joint FAO/WHO Expert Committee on Food Additives in 1961, 1964, 1965, 1973, and 1980 (see Annex I, Refs. 6, 8, 11, 32, 40 and 54). Toxicological monographs were issued in 1961, 1964, 1965, 1973 and 1976 and 1980 (see Annex I, Refs. 6, 9, 13, 33, 41 and 55).

Since the previous evaluations, additional data have become available and are summarized and discussed in the following monograph.

BIOLOGICAL DATA

BIOCHEMICAL ASPECTS

Effects on enzymes and other biochemical parameters

BHT in the diet of Sprague-Dawley rats resulted in a marked decrease in the NADPH-cytochrome P-450 reductase activity of isolated liver microsomal preparations. This effect was not observed when BHT was added *in vitro* to liver microsomes (Rikans et al., 1981). Rats fed 0.4% BHT in their diet showed an increase in GSH-S transferase activity in the liver, but not in lungs and kidneys. GSH-reductase levels were increased in liver and lungs (Partridge et al., 1982). Dietary BHT was also shown to effect the carboxylation process in the conversion of rat liver microsomal protein to prothrombin (Takahashi & Hiraga, 1981).

Addition of cyclic GMP (cGMP added as the dibutyl or 8-bromo form) to BHT suppressed Mishell-Dulton cultures, effected a reversal of the BHT suppression of antibody production (Wess & Archer, 1982).

The observed increase in tumour-specific antigen activity in the colon chromatin of rats treated with 1,2-dimethylhydrazine was abolished by simultaneous treatment with BHT (Gabrelak et al., 1981).

TOXICOLOGICAL STUDIES

Special studies on carcinogenicity

Mouse

Groups each of 100 (B6C3F1) mice, equally divided by sex were fed diets containing 0, 200, 100 or 5000 ppm (0, 0.02, 0.1 or 0.5% of BHT for 96 weeks, followed by a basal diet for six weeks. The diets were made by mixing the BHT with CE-2 (CLEA Japan, Inc., Tokyo) diet at the appropriate concentration and pelleting. At the end of the test period

the surviving animals were killed. A complete autopsy was carried out, and the principal organs and tissues were examined microscopically. Mice that died during the course of the study were also autopsied. In addition terminal blood samples were collected for haematological examination, and serum clinical biochemistry. Urine samples were also examined. During the course of the study, food consumption was similar for test and control groups. Body weights of females in the 1000 and 5000 ppm (0.1 and 0.5% groups were lower than controls, as was the body weight of males in the 5000 ppm (0.5%) group. There were minor changes in the absolute weight of some organs in the high dose groups (salivary glands, heart and kidney). In males the serum GOT and GPT levels in the 5000 ppm (0.5%) group were higher than controls. No other compound related effects were observed in the haematological, serum and urine analysis. Neoplastic lesions were reported in both test and control animals. The tumours that occurred with greatest frequency were adenomas of the lungs, hyperplastic nodules and hepatocellular carcinomas of the liver and malignant lymphomas. However, there was no statistically significant difference between the BHT treated and control groups for the incidence of any type of tumour (Shirai et al., 1982).

Rat

Groups of 57 Wistar rats (seven weeks old) of each sex were maintained on diets containing 2 500 or 10 000 ppm (0.25 or 1% of BHT for 104 weeks. Control groups consisted of 36 rats of each sex. At the end of the test period the surviving animals were killed and a complete autopsy was carried out. The principal organs and tissues were examined microscopically. Terminal blood samples were collected for haematological examination and serum clinical biochemistry. Food intake was similar for test and control animals, but body weight gain was reduced in both male and female rats in the high dose groups. Increased relative liver weight was observed in all test animals, and decreased spleen weight in the females. Total blood cholesterol was increased in all test animals and increased red blood cell counts were observed in females. The overall incidence of tumours was slightly but not significantly higher in BHT treated rats than in controls. The incidence of hyperplastic nodules and of pancreatic carcinomas in female rats and of pituitary adenomas and adenocarcinomas in test animals was higher than those in controls. However, with the exception of the incidence of pituitary adenomas in the low dose females, these differences were not significantly different from controls. Since this effect was not dose related, it was concluded that BHT, under the conditions of this test, was not carcinogenic (Hirose, 1980).

Potentiation or inhibition of carcinogenesis

Groups of Swiss mice were given 1000, 250, or 50 mg/kg urethan or 0.9% NaCl. Seven days later, half the urethan treated animals and half the controls received 300 mg/kg BHT i.p. the remaining animals

receiving corn oil alone. Thirteen weekly injections were given. The number of tumours/lung found 14-24 weeks after the initial urethan doses was significantly increased in the BHT treated animals. In another study, when the interval between injection of the urethan and the first treatment with BHT was delayed for six weeks, BHT treatment

produced more tumours. When the number of BHT injections commencing one week after urethan treatment was reduced from 13 to four, the same significant increase in rumour yield was observed as in the 13-dose study. However, one or two doses of BHT had no significant effect. When the mice were pretreated with 13 injections of BHT, and then treated with urethan one week later, there was no enhancement of tumour yield. Simultaneous administration of BHT and urethan, resulted in fewer rumours compared to animals treated with urethan alone. When mouse strains (C57BL, C3H and BALB/C) which have a low naturally occurring incidence of lung adenoma were treated with urethan and then with multiple injections of BHT, the BHT treatment did not significantly increase rumour incidence or average numbers of rumours per lung (Witschi & Lock, 1979).

Male Strain A mice were injected i.p. with 500 mg/kg urethan, then one week later received repeated injections (one a week for eight weeks) of either 300 mg/kg BHT, or BHA, 500 mg/kg, or Vitamin E, 1000 mg/kg, all dissolved in corn oil. At the termination of the study, only BHT was shown to produce a significant increase in rumour yield. Although the number of rumours produced by BHA treatment was greater than usual, it was not statistically significant. A/J mice treated with 3-methylcholanthrene or dimethylnitrosamine, followed by treatment with BHT (i.p.), resulted in an increase in rumour yield (Witschi et al., 1981). In another study, male A/J mice were injected i.p. with a single dose of urethan and then fed either 0.75% BHT, or BHA or ethoxyquin in the diet, once a week, or continuously for eight weeks. Lung tumours yield was scored four months after the urethan treatment. Dietary BHT, but not BHA or ethoxyquin, under either conditions of the test, enhanced lung rumour formation. Mice were prefed with diets containing either BHA or BHT for two weeks prior to urethan treatment, and then maintained on conventional laboratory diets for four months. The BHT diet had no effect on tumour yield, but the BHA treatment significantly decreased the average number of tumours (Witschi, 1981).

In another study A/J mice were given a single dose of BHT i.p. (400 mg/kg), sufficient to cause acute lung damage and produce cell proliferation in the lung for six to seven days. Urethan was administered continuously by implanted minipumps during this period. Continuous presence of urethan during the period of cell division did not result in an enhanced number of the rumours. When urethan injected mice were dosed i.p. with SKF525A (2-diethylaminoethyl-2-, 2-di-phenylvalerate hydrochloride) and BHT (SKF inhibits lung cell division normally seen following BHT administration), or BHT alone, both treatments gave a very significant increase in lung tumour yield compared to urethan treated controls (Witschi & Kehrer, 1982).

Repeated pulmonary cell division brought about by other treatments, e.g., 95-100% oxygen, were also shown not to enhance tumour development (Witschi & Kehrer, 1982).

Groups of female Sprague-Dawley rats were treated with either 7-12-dimethylbenz[a]anthracene (DMBA) or nitrosomethylurea (NMU) and then fed diets containing 0 or 0.3% added BHT for 30 weeks. Rats treated with DMBA and maintained on the control diet developed 100% tumour incidence (mammary gland) by week 27, whereas, those maintained on the BHT supplemented diet had an incidence of 54% by the end of the

study. Dietary BHT had no effect on the incidence of tumours induced by NMU treatment (King, McCay & Koss, 1981).

Reproduction and behavioural studies

Groups each of 46 rats, six weeks old (Wistar outbred, SPF) were fed diets containing 0, or 0.5 to 0.9% BHT so that the dietary intake of BHT was equivalent to 500 mg/kg during the course of the study. At week 19, the F₀ generation was mated. Twenty-four hours after birth of F₁ rats, the size of the litters was reduced to eight, and half of the litters were cross-fostered. Body weight of parents and offspring and developmental events of offspring were monitored during the course of the study, as well as the reproductive performance of the F₀ rats. Auditory and visual function and locomotive coordination tests were carried out on the F₁ generation. The F₁ animals were autopsied at day 25 of age, and a histological examination made of the brains. Body weights and weight gain of test animals were reduced when compared to controls, and this persisted during gestation. The duration of pregnancy, average body weight, and litter size were similar for test and control animals. The average body weight and weight gain of the F₁ offsprings was significantly reduced in pups nursed by dosed mothers. Pups exposed in utero to BHT also showed a relatively slower development than controls when fostered with non-dosed mothers. Pups exposed to BHT in utero and/or mothers milk showed alterations in the behavioural patterns examined as well as higher incidence in average number of dead cells in the brain (Meyer & Hansen, 1980).

Detailed comments were submitted by the Chemical Manufacturers Association (CMA) (1983) on studies of the effect of BHT on reproduction and teratogenicity. The major comments were concerned with the studies of Brunner et al. (1978) and Vorhees et al. (1981) previously reviewed by JECFA in 1980 as well as the study by Meyer & Hansen (1980).

In the case of the Brunner et al. and Vorhees et al. study, it was concluded that the study showed normal pup survival and development in pups raised by rat dams on diets containing 0.125% BHT. Normal post-weaning development was observed in pups raised by rat dams on diets containing 0.25% BHT, although increased post-weaning mortality occurred in pups raised by dams on the 0.25% and 0.5% diet;

developmental delays occurred in pups in the 0.5% group. In the case of the Meyer and Hansen (1980) study, developmental delays were seen in rats raised by rat dams on diets containing 0.5% BHT. At the 0.25% and 0.5% level, the effect may be due either to toxic effects of BHT on the rat dam, or direct toxicity during lactation. A number of questions were also raised about the design of the Brunner or Vorhees study. These are: (1) the pup selection, in which all litters of fewer than eight live pups were discarded; (2) the excess mortality was reported in terms of pup count rather than affected litters. The data from this study have been audited by the United States FDA (1983). It was concluded that the raw data support the authors observations of increased mortality in the mid-dose and high-dose BHT offspring. However, excess mortality occurred in a limited number of litters; e.g., in the 0.5% group, of the 60 deaths reported in 19 litters, 49 of the deaths occurred in five litters, and in the 0.25% group of the 42 deaths, 21 occurred in two litters, and at the 0.125% level of the

12 deaths, 11 occurred in one litter. It was also noted that in the high dose group; that there was an increased number of litters with eight pups or less, and no litters larger than 12 pups. In the other dose groups, the litter size was comparable to controls.

In the case of the Meyer & Hansen (1980) study, the CMA comments note that the level of BHT used in the study caused toxicity in the dams, which appears directly or indirectly to affect the pups. Reports of teratogenicity studies and/or one generation reproduction studies in several strains of mice and rats as well as a three generation reproduction study in rats were also submitted in the comments to support a "no effect" level of 0.1% BHT in the diet.

Special studies on the effect of BHT on the thyroid

Male MOL/WIST SPF rats, outbred strain (approximately 200 g) were used for the study. BHT was added to a semi-synthetic diet in which the iodine content was controlled at about 12 µg/100 g (nutritional requirement for the rat is 15 µg/100 g). In one study, rats were fed 0, 500 or 5000 ppm (0, 0.05 or 0.5%) BHT in the diet for eight, 26 and 90 days, and the uptake of ¹²⁵I by the thyroid determined. The presence of BHT in the diet resulted in a marked increase in the uptake of ¹²⁵I at all time periods studied. When rats were fed BHT in diets containing varying amounts of iodine (12, 150 or 300 µg/100 g) for 30 days there was a significant increase in thyroid weight in BHT treated animals when compared to controls. BHT in the diet of rats increased liver and thyroid weights at 5000 ppm (0.5%) of the diet, but only thyroid weight at 500 ppm (0.05%). BHT did not change levels of T₃ and T₄ in the blood. The biological half life of thyroxine was increased after 13 days on a BHT diet but returned to normal after 75 days. Electron microscopy of the thyroid glands of rats exposed to dietary BHT (5000 ppm (0.5%)) for 28 days showed an increase in the number of follicle cells (Sondergaard & Olsen, 1982).

Special studies on haemorrhagic toxicosis

The LD₅₀ (i.p.) for BHT showed considerable differences for strains of inbred and non-inbred male mice.

Strain	LD ₅₀ (mg/kg)
DBA/2N (inbred)	138
BALB/cNnN (inbred)	1 739
C57BL/6N (inbred)	917
ICR-JCL (non-inbred)	1 243

In all cases death occurred four to six days after administration of BHT, and was accompanied by massive oedema and haemorrhage in the lung (Kawano et al., 1981).

Male rats (Sprague-Dawley) were fed diets containing 0 or 1.2% BHT for one week. BHT treated rats showed haemorrhages in most organs.

There was a significantly increased leakage of Evans blue into the epididymis. In addition, inhibition of ADP induced platelet aggregation and decreased platelet factor 3 availability was observed. Plasma prothrombin factors were decreased, but fibronolytic activity was unchanged (Takahashi & Hiraga, 1981).

In another study in which the haemorrhagic response was studied in a number of strains of rats (Sprague-Dawley, Wistar, Donryu and Fischer), mice (ICR, ddY, DBA/c, C3H/He, BALB/CaAn and C57BL/6), New Zealand White-Sat rabbits, beagle dogs, and Japanese quail fed diets containing BHT (1.2% of the diet for rats and mice), (1% of the diet for quail), (170 or 700 mg/kg bw for rabbits) and (173, 400 or 760 mg/kg bw for dogs) for a period of 14-17 days. Haemorrhagic deaths occurred among male rats of all strains and female rats of the Fischer strain. Female rats of the Donryu, and Sprague-Dawley strain showed no obvious haemorrhaging. No haemorrhagic effects were noted in rabbits or dogs (Takahashi et al., 1980).

Comments

A recent lifetime study in mice and a 104-week study in rats showed that under the conditions of the test BHT was not carcinogenic.

Additional studies are available on the role of BHT in the enhancement of lung tumour yield by chemical carcinogens, in susceptible species of mice. BHT has also been shown to be effective as a promoting agent in this assay system when the test animals were treated with either polycyclic hydrocarbons or nitrosamine. The lowest

dose at which BHT can act as a promoter of urethan induced lung tumours in the mouse has not been established. No additional studies are available on the possible promotion of hepatic carcinogenesis caused by chemical carcinogens. BHT only acts as a promoter when administered after exposure to the chemical carcinogen, but not before. BHT has also been shown to inhibit the effect of some chemical carcinogens. The protective effect may be associated with changes in the metabolism of the carcinogen resulting from enzyme induction caused by BHT. More information is required on the conditions as well as the mechanisms of the inhibitory or promotional activity of BHT on chemical carcinogens, to assist in interpretation of these studies before they provide a useful basis for the toxicological evaluation.

A recent study on the behavioural and development effects of BHT on rats exposed in utero during lactation showed that BHT caused a significant decrease in body weight gain of both offspring and parent. Altered behavioural patterns, as well as brain lesions were noted in the offspring. However, only one dose level (0.5% BHT in the diet) was used in this study, and this level was toxic to the dams. A detailed analysis of data from the study of Vorhees et al. (1981) showed that at both the mid and high (0.5% and 0.25%) dose levels, the excess mortality may reflect litter effects. The data indicate a "no effect" level for BHT-induced reproductive effects to be 0.1% of the diet of rats. A lifetime feeding study with rats, which involves a single generation reproduction study, is under way. The data from this study will provide additional information to support a "no effect" level from BHT in reproduction studies in the rat.

The haemorrhagic effects of massive doses of BHT seen in certain species of mice but not in dogs and certain species of rats may be related to its ability to interfere with vitamin K metabolism.

Rats exposed to 500 or 5000 ppm (0.05 or 0.5% of dietary BHT) showed a significant increase in thyroid weights, as well as the ability of the thyroid to take up iodine. However, lifetime studies in rats maintained on diets containing up to 10 000 ppm (1%) BHT have not shown adverse effects on the thyroid.

Previously reported studies on induction of microsomal enzymes, reproduction and behavioural effects provide a basis for setting a "no effect" level.

EVALUATION

Level causing no toxicological effect

Mouse: 5000 ppm (0.5%) in the diet, equivalent to 250 mg/kg.
Rat : 1000 ppm (0.1%) in the diet, equivalent to 50 mg/kg.

Estimate of temporary acceptable daily intake for man

0-0.5* mg/kg bw.

FURTHER WORK OR INFORMATION

Required by 1986.

Submission of the lifetime feeding study known to be in progress which includes a single generation reproduction study.

* Group ADI: As BHA, BHT, and TBHQ, singly or in combination.

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See Also:

[Toxicological Abbreviations](#)

[Butylated hydroxytoluene \(BHT\) \(WHO Food Additives Series 15\)](#)

[Butylated hydroxytoluene \(BHT\) \(WHO Food Additives Series 28\)](#)

[Butylated hydroxytoluene \(BHT\) \(WHO Food Additives Series 42\)](#)

[Butylated Hydroxytoluene \(BHT\) \(IARC Summary & Evaluation, Volume 40, 1986\)](#)