2,6-Di-*tert*-butyl-*p*-cresol

(CAS No: 128-37-0)

Health-based Reassessment of Administrative Occupational Exposure Limits

Committee on Updating of Occupational Exposure Limits,
a committee of the Health Council of the Netherlands

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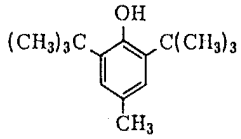
1 Introduction

The present document contains the assessment of the health hazard of 2,6-di-*tert*-butyl-*p*-cresol (BHT) by the Committee on Updating of Occupatonal Exposure Limits, a committee of the Health Council of the Netherlands. The first draft of this document was prepared by MA Maclaine Pont, M.Sc. (Wageningen University and Research Centre, Wageningen, the Netherlands).

The assessment of the toxicity of BHT was mainly based on the reviews by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO96) and the American Conference of Industrial Hygienists (ACGIH) (ACG99). Where relevant, the original publications were reviewed and evaluated as will be indicated in the text. In addition, in May 2000, literature was searched in the databases Toxline, Medline, and Chemical Abstracts starting from 1990, 1990, and 1992, respectively, and using the following key words: BHT, butylated hydroxytoluene, and 128-37-0. The final literature search was carried out in Toxline and Medline in October 2003.

In October 2003, the President of the Health Council released a draft of the document for public review. No comments were received.

2 Identity

name	:	2,6-di-*tert*-butyl-*p*-cresol
synonyms	:	2,6-di-*tert*-butyl-4-methylphenol; 2,6-bis(1,1-dimethylethyl)-4-methyl- phenol; butylated hydroxytoluene; butylhydroxytoluene; dibutylated hydroxytoluene
molecular formula	:	$C_{15}H_{24}O$
structural formula	:	
CAS number	:	128-37-0

3 Physical and chemical properties

molecular weight	:	220.35
boiling point	:	265°C
melting point	:	71°C
flash point	:	127°C (open cup)
vapour pressure	:	at 20°C: 1.3 Pa
solubility in water	:	insoluble (at 20°C: <1 mg/100 mL)
log $P_{octanol/water}$	:	4.17, 5.1 (experimental); 5.03, 5.6, 6.2 (estimated)
conversion factors	:	at 20°C, 101.3 kPa: 1 ppm = 9.2 mg/m^3 1 mg/m^3 = 0.11 ppm

Data from ACG99, BUA94, IARC86, NLM03, http://esc.syrres.com.

BHT is a white, crystalline, odourless solid. BHT is easily oxidisable and can act as a radical scavenger (ACG99, BUA94).

4 Uses

BHT is widely used as a stabiliser and antioxidant for ground vehicle and aviation gasolines; lubricating, turbine, and insulating oils; and waxes, synthetic and natural rubbers, paints, plastics, and elastomers. Highly purified grades are suitable for use in foods to retard oxidation of animal fats, vegetable oils, and oil-soluble vitamins. It is also used in food packaging materials such as waxed papers, paperboard, and polyethylene. It is important in delaying the onset of rancidity of oils and fats in animal feeds and in preserving the essential nutrients and pigment-forming compounds of these foods (ACG99).

In the Netherlands, BHT is allowed as antioxidant in fats and oils and in chewing gum at levels of 100-400 mg/kg (Ano99). In the US, regulations permit the use of BHT as a food additive in certain potato products, rice, margarine, breakfast cereals, chewing-gum, and certain meat products at specified levels ranging from 0.001-0.1% and in food packaging material in the USA (IARC86).

5 Biotransformation and kinetics

Human data

Male human volunteers (n=4), who were given a single oral dose of approximately 40 mg ^{14}C-labelled BHT, excreted about 50% of the dose in the

urine within 24 hours and 75% at the end of the observation period (11 days). BHT was biotransformed by oxidation of the 4-methyl group in the ring and of one of the *tert*-butyl methyl groups. Free and conjugated 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (BHT-COOH) were minor urinary components (Dan68). However, in 2 other studies, in which human volunteers received single oral doses of 100 or 1000 mg of BHT, free and conjugated BHT-COOH were identified as major metabolites (Hol70). In a more recent study, a furan derivative, 5-carboxy-7-(1-carboxy-1-methylethyl)-3,3-dimethyl-2-hydroxy-2,3-dihydroxybenzofuran, was identified as a main human metabolite (Wie78) (see Figure1a, Annex I). In another study, 7 male volunteers were given an oral dose of BHT of 0.5 mg/kg bw. The peak BHT level in plasma was reached 90 minutes after dosing. Large variations were seen in BHT plasma concentrations (mean: 0.09 mg/L). BHT-COOH (mainly conjugated) was excreted in amounts of 2.8% of the dose within 2 days (Ver89).

Animal data

After a single application of 5 mg ^{14}C-labelled BHT in a lipophilic vehicle to the unoccluded skin of guinea pigs (n=5; application area: 1.66 mg/cm^2), an average of 8.5% of the applied dose was excreted in the urine within 8 days. Most of he radioactivity (63%) was excreted within 24 hours after application. Washing of the skin, 24 hours after application, reduced the excretion to 5.4% of the applied dose. Courtheoux et al. conducted also a repeated dermal study, in which guinea pigs received daily doses of 5 mg BHT (1.66 mg/cm^2) for 21 days. On days 1, 8, and 15 only, the animals were dosed with 5 mg ^{14}C-BHT. The amounts of radioactivity excreted in urine were 4.4, 7.9, and 9.1% of the dose, within 7 days after each radiolabelled application. About 35% of the radioactivity was excreted within 24 hours after application. Washing of the skin, one hour before application of radiolabelled BHT, had no significant influence on the amount of absorption (Cou85).

After a single oral dose of ^{14}C-BHT of 1 mg to rats, 24 and 37% was excreted in the urine and 42 and 35% in the faeces in males and females, respectively, within 4 days (Dan65). In a later study, following single intragastic doses of ^{14}C-BHT of 20 or 500 mg/kg bw, 9-12 and 47-38% of the radioactivity were excreted in the urine and the faeces, respectively, 24 hours after administration, amounting to 16-69 and 70-64%, respectively, after 3 days (Mat84). BHT residues were found in the fat and the liver of rats, given approximately 250 mg/kg bw/day BHT via the food for 35 days. The half-life of elimination of BHT from these tissues was 7 to 10 days (Dan65). In mice, approximately 75% of a

single oral dose was excreted in the urine during the first 24 hours. This was followed by a slower phase during which an additional 10% was excreted over the next 4 days. The total amount found in the faeces was less than 1% of the dose (Dau80). In another study in mice, 41-65% of the radioactivity was excreted in the faeces, 26-50% in the urine, and 6-9% in expired air within 24 hours after administration of single oral doses of ^{14}C-BHT of 20 or 500 mg/kg bw. The half-life of elimination of radioactivity from the major tissues studied was 9-11 hours. However, when daily doses were given for 10 days, the half-life was 5-15 days (Mat84). Guinea pigs that were given a single oral dose of 5 mg ^{14}C-BHT excreted 67% of the radioactivity in urine and 18% in the faeces within 7 days. Most of the urinary radioactivity (approximately 50% of the dose) was excreted in the first 24 hours urine. When given by the intravenous route, 60% of radioactivity was excreted in the urine and 15% in the faeces within 7 days (Cou85).

Mice of the ddY strain responded to an intraperitoneal injection of BHT of 400 mg/kg bw with a significant reduction in the glutathione content of lung tissue, but not of the liver or kidney tissue (Mas84). BHT binds to mouse lung tissue to a greater extent than to liver and kidney tissue, and more is bound in mouse than in rat tissues (Keh80).

In rats, the predominant metabolic pathway involves oxidation of the 4-methyl group, yielding BHT-COOH (both free and glucuronidated) as a major metabolite, and 3,5-di-*tert*-butyl-4-hydroxybenzyl alcohol and benzaldehyde (BHT-OH and BHT-CHO) as minor metabolites. In addition, the mercapturic acid *S*-(3,5-di-*tert*-butyl-4-hydroxybenzyl)-*N*-acetylcysteine (BHT-mercapturic acid) was detected as a major metabolite (Dan68, Lad67). This mercapturic acid may be formed by reaction of BHT-quinone methide (2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexadienone) with glutathione. This reactive BHT-quinone methide has been identified in the liver and bile of rats (Taj81, Tak79) and may be responsible for BHT-induced lung damage or hepatotoxicity in mice (Keh85, Miz87). Metabolites produced in mice are similar to those produced in rats, except that the major biotransformation in mice was by oxidation of the *tert*-methyl groups (Mat84). In rabbits, orally administered BHT was metabolised to BHT-OH, BHT-COOH, and BHT-dimer. The urinary metabolites of BHT comprised 38% as glucuronides, 17% as sulphates, and 7% as free phenols. Unchanged BHT was present only in the faeces (Aka62).

The confirmed and tentative metabolic pathways are presented in Figures 1a and 1b (see Annex I).

6 Effects and mechanism of action

Human data

In a study on workers ('not less than 15 individuals') in the rubber and plastic industries, BHT was mildly irritating to the skin and a skin sensitiser in 29 and 19% of the subjects, respectively (Mal52). In a study on eczema patients, 2 out of 112 were positive in a patch test with 2% BHT (Roe76). In another study, 7 patients with chronic asthma or chronic vasomotor rhinitis showed an exacerbation of respiratory symptoms following oral intake of 125 or 250 mg BHT plus butylated hydroxyanisole (BHA) (Fis73). Patch testing with BHT did not produce any positive reaction in 1336 eczema patients (Fly90). When 358 patients from a dermatology clinic were patch tested with 2% BHT, none had an allergic reaction and 2 had an irritant reaction (Kan99). Double-blind, placebo-controlled challenge tests with a 1:1 mixture of BHT and BHA (50 mg) were carried out in 44 patients with chronic urticaria, in 91 with atopic dermatitis, and in 123 with contact dermatitis. No positive reactions were seen (Han86). In very rare cases, the intake of food containing BHT resulted in urticaria, a food-intolerance reaction (And97).

Two cases of intoxication have been reported following incidental oral intake of high doses of BHT. In one case, a woman who ingested 4 g of BHT developed stomach cramp, general weakness, nausea and vomiting, followed by fatigue, mental disorientation, and short-term unconsciousness. A few days after the intake, the symptoms had disappeared (Shl86). In the other case, a woman who ingested 80 g BHT developed neurological symptoms within one hour, which were fully reversible (Gro86).

Animal data

Irritation and sensitisation

BHT was slightly irritating to rabbit eyes (McO49, Bee76).

When a dose of BHT of 420 mg/kg bw in ether was applied to the skin of rabbits, slight irritation was noted (McO49). This was confirmed in a later conducted patch test (Bee76). BHT had no skin-sensitising activity in guinea pigs following repeated application of the compound (Dei55). In a maximisation test with guinea pigs, animals sensitised to 2-methylol *p*-*tert*-butylphenol and

2,6-dimethylol *p-tert*-butylphenol did not show cross-reactions to a 13.3% solution of BHT (11/23 and 1/24 animals sensitised, respectively) (Zim98).

With respect to the respiratory tract, the sensory irritation in the upper part was studied by determining the concentration associated with a 50% decrease in respiratory rate (RD_{50}). After exposing male Swiss Webster mice (n=4/group) to vapour concentrations between 1.9 and 5.1 ppm (18-47 mg/m^3) for 30 minutes, an RD_{50} for sensory irritation of 3.6 ppm (33 mg/m^3) was calculated. None of the mice exhibited breathing patterns of pulmonary irritation (Sta97).

Acute toxicity

Results of acute lethal toxicity studies of BHT are summarised in Table 1.

Table1 Summary of acute lethal toxicity studies of BHT in experimental animals.

exposure	species (sex)	LD_{50} (mg/kg bw)	reference
oral	rat (male)	1700	Dei55
	rat (female)	1970	Dei55
	rat (male)	1906	BUA94
	rat (female)	2255	BUA94
	rat (male, female)	>10,000[a]	Spa78
	rat (male, female)	2450	Kar59
	rat	2250	LSR73
	rat	5800	BUA94
	mouse (male)	1040	McO49
	mouse	1800	LSR73
	mouse	1350	BUA94
	mouse	2000	Kar59
	rabbit	2100-3200[b]	Dei55
	rabbit	3200	LSR73
	guinea pig	10,700[b]	Dei55
	cat	940-2100[b]	Dei55
intravenous	mouse	180	NIO03
intraperitoneal	rat	8000	BUA94
	mouse (male)	138-3550[c]	Kaw81, Yam80

[a] By gavage as 33% suspension in propylene glycol.
[b] Approximate lethal dose.
[c] Depending on mouse strain.

The intraperitoneal LD_{50}s for BHT showed considerable strain-dependent differences among (male) mice.

At high doses, symptoms of poisoning were salivation, miosis, excitation, tremor, and signs of paralysis. Toxic signs before death included reduced body

weight, breathing difficulties, coma, and enlarged lungs with pulmonary oedema and haemorrhages (BUA94).

The effect of single oral dose of BHT on blood coagulation factors II (prothrombin), VII, IX, and X was studied in Sprague-Dawley rats, given 200, 400, or 800 mg/kg bw. At 800 mg/kg bw, levels of coagulation factors II, VII, and X were reduced 48 hours after treatment (NOAEL: 400 mg/kg bw). Factor IX decreased in a dose-dependent fashion (Tak87). According to the committee, this indicates that the haemorrhages observed at high doses of BHT, leading to mortality, are due to inhibition of blood coagulation.

In mice, a single intraperitoneal dose of BHT of 400 mg/kg bw caused pulmonary alveolar cells Type I necrosis and proliferation of alveolar cells Type II (Ada77). BHT-induced lung damage is associated with increased lung weight and biochemical changes, such as increases in DNA, RNA, and protein synthesis (Sah75), cyclic GMP levels (Kuo78), and protein phosphorylation (Mal79). Four strains of mice, with intraperitoneal LD_{50}s ranging from 350-1700 mg/kg bw, developed similar levels of lung injury at equivalent doses, but no correlation could be made between lung injury and lethality (Keh90).

Sprague-Dawley rats, given a single oral (gavage) dose of BHT of 500 mg/kg bw, did not show changes in serum levels of liver enzymes (ALAT, ASAT, alkaline phosphatase, or LDH) and of albumin. A slight reduction in the concentration of clotting factor IX was observed. There was no evidence of hepatocellular necrosis. However, mitotic activity of hepatocytes was increased, as well as hepatic epoxide hydrolase, ethoxyresorufin-*O*-deethylase (EROD), and ethoxycoumarin-*O*-deethylase (ECOD) enzyme activities, indicating hepatic enzyme induction (Pow91). When male F344 rats were given a single oral dose of 1000 mg BHT/kg bw, both renal injury (as demonstrated by proteinuria and enzymuria) and liver injury were observed. More marked effects were seen in rats pre-treated with phenobarbital (Nak88).

Short-term toxicity

In a dermal study, CD1 mice (n=10/sex/group) received 3 applications per week of 0.1% DMSO solutions containing 0, 5, 10, 20, 30 mg BHT, corresponding to BHT doses of 0, 140, 289, 678, or 867 mg/kg bw/day for males and 0, 208, 415, 830, and 1245 mg/kg bw/day for females, for 4 weeks. Clinical signs were respiratory distress, lethargy, and weight loss, which started after the second application in male and female animals of the 20- and 30-mg groups and after the third application in most of the females of the 10-mg group and in one male and female of the remaining groups. A dose-related increased mortality occurred

mostly between the 4th and 8th day in 0/10, 1/10, 7/10, 8/10 males and 1/10, 9/10, 10/10, and 10/10 females of the 5-, 10-, 20-, and 30-mg group, respectively. Macroscopic and microscopic examination showed congestion and enlargement of the lung and damage of alveolar type I-cells. The NOAEL for males was 140 mg/kg bw/day and the LOAEL for females 208 mg/kg bw/day, the lowest dose tested. In a similar study in male and female rats and male hamsters, at dose levels of about 2000, 2300, and 3100 mg/kg bw/day, respectively, only slight growth retardation in male rats and hamsters was observed. At post-mortem gross and microscopic examinations, there were no remarkable treatment-related changes (Miy86).

Groups of male Wistar rats were given oral (gavage) doses of BHT of 0, 25, 250, 500 mg/kg bw/day (n=10/group) for 28 days. At the high dose, progressive periportal hepatocyte necrosis of the liver was observed and at 500 and 250 mg/kg bw, a dose-related hepatomegaly. The NOAEL for liver injury was 25 mg/kg bw/day. Biochemical changes consisted of dose-related induction of epoxide hydrolase and reduction of glucose-6-phosphatase activity. BHT accumulated in fat in a dose-dependent fashion, but not in the liver (Pow86).

In an old (1961) oral study, rats (n=3/sex/group) were given BHT via the diet, containing a 20% lard supplement, at doses equivalent to approximately 0, 50, 100, 150, 200, or 250 mg/kg bw/day, for 6 weeks. At 150 mg/kg bw/day and above, a reduced growth rate was observed and at 100 mg/kg bw/day and above, the absolute and relative liver weights were increased in both sexes and the relative adrenal weight in males. Microscopic examination did not show abnormalities. A dose-related increase in cholesterol levels was observed at all dose levels. The NOAEL for increased liver weight was 50 mg/kg bw/day (FAO96).

In a more recent study, groups of F344 rats (n=5/sex/group) were fed BHT via the diet at dose levels equivalent to approximately 310, 625, 1250, or 2500 mg/kg bw/day, for 7 weeks. All animals in the high-dose group and one animal in the 1250 mg/kg bw group died. A dose-related decrease in body weight was observed, with animals in the 1250 mg/kg bw group weighing only 38 to 44% of control values. At this dose, a slight increase in haematopoiesis was also observed in both sexes. The LOAEL for reduced body weight was 310 mg/kg bw/day (NCI79).

Groups of male ddY mice (n=10/group) were given BHT via the food at levels equivalent to 0, 1570, 1980, 2630, 3370, 4980, or 5470 mg/kg bw/day, for 30 days. Body weights and absolute kidney weights were decreased and relative kidney weights increased in a dose-related fashion. Microscopic examination

revealed a dose-related increase in toxic nephrosis, as indicated by tubular degeneration, necrosis dilatation, and cysts. No treatment-related changes were observed in the liver. The LOAEL for renal lesions and body weight reduction was 1570 mg/kg bw/day (Tak92).

B6C3F_1 mice (n=5/sex/group), given doses equivalent to approximately 465, 930, 1875, 3750, or 7500 mg/kg bw/day for 7 weeks, showed dose-related mortality at the 2 high-dose levels and a dose-related decrease in body weight at all dose levels. Microscopic examination revealed vacuolisation of hepatocytes in animals at 3750 mg/kg bw/day. The NOAEL was 1875 mg/kg bw/day (NCI79).

In another study, groups of B6C3F_1 mice (n=10/sex/group) were given BHT at dietary doses equivalent to approximately 375, 750, 1500, 3000, or 6000 mg/kg bw/day, for 10 weeks. In the high-dose group animals, body weight gain was significantly reduced. Microscopic examination revealed atrophy of the spleen, heart, and kidneys. No liver or lung damage was found. The NOAEL was 3000 mg/kg bw/day (Ina88).

Groups of C3H mice (n=26-46/sex/group) were given BHT via the diet at doses equivalent to 0, 39, or 390 mg/kg bw/day, for 10 months. Treatment-related decreases in body weights were seen at both levels. In males, a significantly increased incidence of hepatocellular adenomas was found in treated groups compared with controls, which was more pronounced at the low dose. No significant differences in lung tumours were found between treated and control groups (Lin86). A group of 18 male BALB/C mice, given BHT at a dietary level equivalent to 1125 mg/kg bw/day for 12 months, developed hyperplasia of the hepatic bile ducts with an associated subacute cholangitis (Cla73).

Administration of 50 or 500 mg/kg bw BHT by gastric intubation to rhesus monkeys for 4 weeks produced no increase in liver weight or liver DNA, RNA, or protein content. The hepatocytes of monkeys given the high dose showed moderate proliferation of smooth endoplasmatic reticulum. Biochemically, an induction of the liver enzyme *p*-nitroanisole-*O*-demethylase was observed, which increased with time. No abnormalities were observed in clinical chemical liver function or electrolyte tests, in haematology, or in microscopic examination of other organs than the liver (All72).

Feeding BHT to mice and rats also resulted in an increased activity of liver enzymes and increased liver weights. For example, giving BHT to female Wistar rats via the diet at doses equivalent to approximately 5, 50, 500, or 2500 mg/kg bw/day for 28 days, produced a dose-related increase in smooth endoplasmatic reticulum in hepatocytes and of liver aminopyrine demethylase activity, which

returned to normal within 10-20 days after discontinuation of BHT dosing. The NOAEL was 5 mg/kg bw/day (Bot70). Other studies showed increased activities in hepatic microsomal epoxide hydrolyse and glutathione-S-transferase in BHT-treated Swiss mice (Ham83) or in activities of hepatic microsomal biphenyl-4-hydroxylase (Cre66, Lak76), cytochrome P-450 (Bur72), ethyl morphine N-demethylase (Lak76), and glutathione-S-transferase (Par82) in BHT-treated rats. In a more recent study, Wistar rats given a dietary BHT level of approximately 100 mg/kg bw/day, for 30 days, showed statistically significant increased liver weights, hepatic microsomal lipid peroxidase activity, hepatic phospholipids, and serum HDL cholesterol, compared to a control group (Yam95).

Feeding of male ICR mice at approximately 75 or 750 mg/kg bw/day for 12 months, resulted in statistically significant increased liver weights at the high dose, compared with a control group. Other organs were not affected. At both dose levels, enhanced activities were observed for intestinal glutathione *S*-transferase and hepatic glutathione peroxidase, while hepatic glutathione *S*-transferase was increased at the high dose only. No changes were observed in clinical chemical liver and kidney function tests and in serum cholesterol levels (Jan99).

In several studies, the effects of BHT on blood clotting were investigated. Groups of male Sprague Dawley rats (n=10/group) were fed BHT at doses equivalent to approximately 450, 525, 600, 750, 900, or 1050 mg/kg bw/day for 40 days. A dose-related effect on mortality was observed at levels of 525 mg/kg bw/day or more during the period from 9 to 37 days. Spontaneous massive bleeding to the pleural and peritoneal cavities and external haemorrhage was observed in all dead animals. The prothrombin index (i.e., the ratio between the prothrombin times of control and BHT-treated animals) of surviving animals was decreased, dependent on BHT dose. At the lowest dose, the decrease was approximately 65% (Tak78a). In a follow-up study, the same strain of rats were given dietary levels of 7.5, 14.7, 34.1, 62.5, 129, 227, or 529 mg/kg bw/day of BHT for 1 week, or 6.7, 13.4, 29.9, 52.9, 96, 230, or 326 mg/kg bw/day for 4 weeks. A significant decrease (14%) of the prothrombin index was already seen at 14.7 mg/kg bw/day, after one week of BHT administration ($p < 0.05$). However, feeding for 4 weeks caused a significant decrease on the prothrombin index (21%) at the top dose only (Tak78b).

Male albino rats given 3 consecutive daily doses of 380, 760, or 1520 mg BHT/kg bw/day did not show haemorrhages. However, a dose-related increase in prothrombin time (i.e., a decrease in prothrobin index) was observed at the 2 high-dose levels (Kra84). Male Sprague-Dawley rats receiving approximately

190 mg BHT/kg bw/day for 2 weeks had decreased concentrations of vitamin K in the liver and inceased levels in the faeces. It was suggested that the vitamin K deficiency induced by BHT might be due to effects of BHT on absorption and excretion of vitamin K (Suz83). This was further investigated in a study, in which groups of Sprague-Dawley rats were given BHT at a dose of approximately 1000 mg/kg bw/day for 1 to 7 days. Vitamin K-dependent coagulation factors II (prothrombin), VII, VIII, IX, and X were significantly reduced in a time-dependent fashion when BHT was administered for 2 to 7 days. Haemorrhages occurred in epididymis of rats given BHT for 4 to 7 days (Tak86).

In another study, the effect of BHT on blood coagulation was investigated in rats, receiving BHT in vitamin K-sufficient and vitamin K-supplemented diets. Dosing of 3000 mg/kg bw/day for up to 21 days in a diet containing a minimum of 3 ppm vitamin K_3 caused decreased levels of individual vitamin K-dependent clotting factors but had no effect on overall prothrombin time (involving factors II, V, VII, X, and fibrinogen). The effects on individual vitamin K-dependent clotting factors were prevented if rats were given BHT (3000 mg/kg bw/day) in diets supplemented with a further 250 ppm vitamin K_3. In another 28-day experiment, the prothrombin time, measured with a more sensitive thrombotest, was prolonged in rats receiving 600 mg BHT/kg bw/day via the diet, containing 3 ppm vitamin K_3. No effect on prothrombin time was measured at BHT doses of 12.5 or 125 mg/kg bw/day. The effect at 600 mg/kg bw/day was prevented by concurrent dietary supplementation with a further 3 ppm vitamin K_3. The NOAEL for effects on vitamin K-dependent blood clotting factors, in rats given diets supplied with recommended amounts of vitamin K, was 125 mg/kg bw/day (Cot94).

In a study to investigate the strain and species dependency of haemorrhages following BHT feeding, a number of strains of rats of both sexes (Sprague-Dawley, Wistar, Fischer), male mice (ICR, ddY, C3H/He, BALB/CaAn, C57BL/6), male New Zealand White rabbits, and male Beagle dogs were fed BHT at doses up to 1120 mg/kg bw/day for 3 weeks (rats), 1925 mg/kg bw/day for 1 week (mice), 390 mg/kg bw/day for 2 weeks (rabbits), and 760 mg/kg bw/day for 2 weeks (dogs). Male guinea pigs and male golden hamsters received intraperitoneal injections of up to 380 mg/kg bw/day and 760 mg/kg bw/day, respectively, for 3 days. Haemorrhagic deaths occurred among male rats of all strains and in female rats of the Fischer strain. Epididymal haemorrhages were observed in ddY mice and cerebral haemorrhages in some guinea pigs. No effects were noted in the other species. The prothrombin index was significantly

reduced in all strains of rats (both sexes) and mice (except ICR strain) but no significant changes were noted in the other species (Tak80).

In another study, haemorrhagic deaths were not observed in ddY male mice given BHT via the diet at levels ranging from 1570 to 5470 mg/kg bw/day for 30 days or in guinea pigs fed 85 to 660 mg/kg bw/day for 14 to17 days. Prothrombin times were significantly increased in mice only, probably because of liver damage rather than by interference of BHT or a metabolite with vitamin K (Tak92).

The results of the above short-term toxicity studies are summarised in Table 2.

Table 2 Summary of short-term toxicity studies of BHT.

exposure route	species (strain)	dose levels (mg/kg bw/day)	exposure duration	critical effect	NOAEL (mg/kg bw/day)	reference
dermal	mouse (CD1)	140-867 (males) 208-1245 (females)	4 weeks	lung injury	140 (males) LOAEL: 208 (females)	Miy86
	hamster	2000 (males) 2300 (females)	4 weeks	not identified	2000 (males) 2300 (females)	Miy86
oral	rat (Wistar)	0-500	4 weeks	liver injury	25	Pow86
	rat (Wistar)	0-2500	4 weeks	liver enzyme induction	5	Bot70
	rat (Wistar)	0-100	4 weeks	liver weight	LOAEL: 100	Yam95
	rat (not given)	0-150	6 weeks	liver weight	50	FAO96
	rat (F344)	0-2500	7 weeks	body weight	LOAEL: 310	NCI79
	rat (Sprague-Dawley)	0-1050	40 days	blood coagulation	LOAEL: 450	Tak78a
	rat (Sprague-Dawley)	7.5-529	7 days	blood coagulation	7.5	Tak78b
	rat (Sprague-Dawley)	6.7-326	4 weeks	blood coagulation	230	Tak78b
	rat (Sprague-Dawley)	0-600	4 weeks	blood coagulation	125	Cot94
	monkey (rhesus)	0-500	4 weeks	liver enzyme induction	50	All72
	mouse (ddY)	0-5470	4 weeks	renal injury	LOAEL: 1570	Tak92
	mouse ($B6C3F_1$)	0-7500	7 weeks	liver injury	1875	NCI79
	mouse ($B6C3F_1$)	375-6000	10 weeks	body weight	3000	Ina88
	mouse (C3H)	0-390	10 months	body weight	LOAEL: 39	Lin86
	mouse (BALB/C)	1125	12 months	liver injury	LOAEL: 1125	Cla73
	mouse (ICR)	0-750	12 months	liver enzyme induction	LOAEL: 75	Jan99
	mouse (ddY)	0-5470	4 weeks	blood coagulation	LOAEL: 1570	Tak92
	dog (Beagle)	0-760	2 weeks	blood coagulation	760	Tak80
	guinea pig	0-2000	2 weeks	blood coagulation	660	Tak92

Long-term toxicity and carcinogenicity

Groups of Fischer 344 rats (n=50/sex/group; controls: n=20/sex) were given BHT (purity: 99.9%) at dietary doses equivalent to 0, 150, or 300 mg/kg bw/day, for 105 weeks. There was no significant treatment-related effect of BHT on mortality, but body weights were decreased at both dose levels. Gross and microscopic examination of organs did not show statistical significant increases in the incidence of any tumour when compared to controls. In female rats, there was a dose-related statistically significant decrease in the incidence of adenomas of the pituitary. Apart from an increased incidence in focal alveolar hystiocytosis in the female animals of the low- and high-dose group (12/48 and 21/48, respectively, vs. 2/18 in controls), no (possibly) treatment-related non-neoplastic lesions were found in any of the groups. The LOAEL was 150 mg/kg bw, based on reduced body weight (NCI79).

In another study, Wistar rats (n=57/sex/group) were given BHT (purity: not specifed) via the diet at doses equivalent to 0, 125, or 500 mg/kg bw/day, for 104 weeks. At termination of the study, 65% of the control and >72% of treated rats were still alive. Treatment-related effects were a decrease in body weight gain at the high dose, increases in absolute and relative liver weights in animals of both groups, and increased GGT levels in males of both groups. Gross and microscopic examination showed a statistically significant increased incidence of pituitary adenomas in the low-dose, but not in high-dose female animals (6/46 and 3/51, respectively, vs. 0/32 in controls). Since this effect was not dose-related, Hirose et al. concluded that the tumours observed did not seem to be induced by BHT treatment. The LOAEL was 125 mg/kg bw/day (Hir81).

Groups of male F344 rats (n=21/group; controls: n=36) were fed BHT (purity: not specified) at doses equivalent to 0, 7.7, 23, 75, 225, or 450 mg/kg bw/day for 76 weeks. In a second experiment, 27 animals received 900 mg/kg bw/day for 110 weeks. At 225 mg/kg bw/day and above, body weights were decreased and at 450 mg/kg bw/day, absolute and relative liver weights were increased compared with controls. Gross and microscopic examination revealed a decreased incidence of pre-neoplastic liver foci in rats receiving 900 mg/kg bw/day. Hepatocellular adenomas were found in all groups, with no treatment-related trend in incidence. No statistically signicant differences were found in the incidence of grossly observable tumours in specific organs. The NOAEL was 75 mg/kg bw/day (Wil90b).

In a 2-generation long-term toxicity study, groups of Wistar SPF rats (n=40-60/sex/group) were given BHT (purity:>99.5%) via the diet at doses equivalent to 0, 25, 100, or 500 mg/kg bw/day for 13 weeks, after which time the males and

females within each group were mated. Females were maintained on treatment throughout pregnancy and the lactation period (see Section 'Reproduction toxicity'*).* Groups of offspring (F1) of each sex (n=80-100/sex/group) were continued on the same treatment, except that the high-dose level was reduced to 250 mg/kg bw/day, due to renal toxicity in the parents. The dosing was terminated when the progenies were 141-144 weeks of age. There was dose-related increase in survival in all treated groups. At 2 years of age, survival rates in the high-dose group were 86% in both male and female rats vs. 70 and 69% in male and female controls, respectively; at study termination, these figures were 44 and 39% for high-dose males and females, respectively, vs. 16 and 17% in the corresponding control groups. Body weights were reduced in all treated groups in a dose-related fashion. No changes were observed in haematological parameters. Microscopic examination revealed increased incidences of liver adenomas (males: 1/80, 5/80, and 18/99 in low-, mid-, and high-dose group, controls: 1/100; females: 3/79, 6/80, and 12/99, respectively, vs. 2/100) and carcinomas (males: 0/80, 1/80, and 8/80, respectively, vs. 1/100; females: 0/79, 0/80, and 2/99, respectively, vs. 0/100). The first adenoma and carcinoma in treated animals were seen in high-dose males at week 115 and week 132, respectively, while all carcinomas and most of the adenomas were found at termination. In the high-dose males, there was also one haemangioendotheliosarcoma. Non-neoplastic lesions included a dose-related increased incidence of bile duct proliferation and cysts in males and focal cellular enlargement in females. The LOAEL was 25 mg/kg bw/day, based on body weight loss. The NOAEL for liver carcinomas was 100 mg/kg bw/day (Ols86). The committee is of the opinion that, due to the large differences in survival time between treatment and control groups, it is difficult to draw conclusions about the significance of the observed tumour incidences of the liver in the treated groups.

Groups of BALB/c mice (n=100 males, 50 females) were given dietary doses of BHT equivalent to 0 or 1125 mg/kg bw/day, for 18 months. Mortality was low in both groups (>90% survived the end of the study). No difference was observed in the incidence of lung tumours or reticulum cell sarcomas between treated and control mice. No liver tumour, thymic lymphoma, myeloid leukaemia, or tumour of the forestomach was seen in BHT-treated mice (Cla78).

In another study, groups of $B6C3F_1$ mice (n=50/sex/group; controls: n=20/sex) received BHT (purity: 99.9%) via the diet at doses equivalent to 450 or 900 mg/kg bw/day, for 107 or 108 weeks. Dose-related decreases in mortality and body weight were observed. Statistically significant increases in the incidence of alveolar/bronchiolar adenomas or carcinomas were found in

females of the low-dose, but not of the high-dose group (16/46 and 7/50, respectively, vs. 1/20 in controls). No differences in the incidence of any other tumour were seen between treated and control groups. Significant non-neoplastic effects comprised dose-related increases in hepatocytomegaly, peliosis, cellular degeneration, and cytoplasmic vacuolation of the liver in male rats (NCI79).

Groups of B6C3F_1 mice (n=50-52/sex/group) were given BHT (purity: not specified) via the diet at dose levels equivalent to 0, 30, 150, or 750 mg/kg bw/day, for 96 weeks, and were maintained on basal diet for a further 8 weeks. Survival at termination of the study was >60% in all groups. Body weights of females in the mid- and high-dose groups and of males in the high-dose group were lower than controls. At the high dose, serum ALAT and ASAT were increased, but no other compound-related effects were observed in the haematological and biochemical analysis in serum and urine or in gross or microscopic examination of organs. There were no statistically significant differences in the incidence of neoplasmas between BHT-treated and control groups (Shi82).

BHT (purity: not given) was administered to B6C3F_1 mice (n=50/sex/group) via the diet, at concentrations 0, 1640, or 3480 mg/kg bw/day (males) or 0, 1750, or 4130 mg/kg bw/day (females), for 104 weeks, and were maintained on basal diet for a further 16 weeks prior to pathological examination. A dose-related decrease in body weight was observed in both sexes. Survival at the end of the study was increased with dose. In male mice, there was a statistically significant dose-related increase in hepatocellular adenomas and in foci of altered hepatocytes. The incidence of hepatocellular carcinomas was not statistically significantly different between treated and control groups (Ina88).

The results of the above long-term toxicity studies are summarised in Table 3.

Table 3 Summary of long-term oral toxicity studies of BHT.

species (strain)	dose levels (mg/kg bw/day)	exposure duration	critical effect	NOAEL (mg/kg bw/day)	reference
rat (F344)	0-300	105 weeks	body weight	LOAEL: 150	NCI79
rat (Wistar)	0-500	104 weeks	liver weight	LOAEL: 125	Hir81
rat (F344)	0-900	110 weeks	body weight	75	Wil90b
rat (Wistar)[a]	0-250	144 weeks	body weight	LOAEL: 25	Ols86
mouse (BALB/c)	0, 1125	18 months	tumours	1125	Cla78
mouse ($B6C3F_1$)	0-900	107 weeks	liver injury	LOAEL: 450	NCI79
mouse ($B6C3F_1$)	0-750	96 weeks[b]	body weight	30	Shi82
mouse ($B6C3F_1$)	0-3480 (males) 0-4130 (females)	104 weeks[c]	liver injury	LOAEL: 1640	Ina88

[a] Second generation of a 2-genereation reproduction toxicity study.
[b] Followed by an 8-week exposure-free period.
[c] Followed by a 16-week exposure-free period.

BHT was studied in mice and rats for its ability to modify the carcinogenicity of selected chemical agents. When administered with known carcinogens, BHT enhanced, inhibited, or had no effect on carcinogenicity (IARC86). In a range of studies, it was demonstrated that BHT administration before or simultaneously with the carcinogen evidently had a protective influence, whereas a subsequent BHT administration had a promoting effect (IARC86, BUA94, FAO96).

Mutagenicity and genotoxicity

Mutagenicity assays comprised tests for the detection of gene mutations in bacteria (*in vitro* and host-mediated assays), mammalian cells, and *Drosophila (in vitro)* and for cytogenicity in mammalian cells (*in vitro* and *in vivo*), e.g., sister chromatid exchanges (SCE), structural chromosomal effects (chromosome aberrations and micronucleus formation), numerical chromosome effects (polyploidy), and dominant lethal mutations (*in vivo*), and other genotoxicity assays, e.g., tests for DNA damage/repair (*in vitro*) and DNA binding (*in vivo*).

- *In vitro* tests:
  - Gene mutation assays. Tests for reverse mutations in several strains of *S. typhimurium* (TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, TA1538), and *E. coli* were negative when tested at concentrations up to 10 mg/plate, in the absence or presence of a rat liver S9 metabolic activation system (Bru75, Det93, Hag88, Kin81, Wil90a, Yos90).
    BHT was negative in a host-mediated assay in mice, given BHT as single

oral doses of 30, 900, or 1400 mg/kg bw or as repeated doses of 30, 250, or 500 mg/kg bw/day, for 5 days, using *S. typhimurium* G46 and TA1530 as markers (Bom02, BUA94, FAO96).
BHT did not induce gene mutations in the HPRT forward mutation assay in cultured rat liver epithelial cell lines at 60-90 µg/mL, in the absence of a metabolic activation system (Wil90a). However, positive results were obtained in the HPRT assay in cultured V79 Chinese hamster cells and in the $TK^{+/-}$ assay in cultured L5178Y mouse lymphoma cells but only after metabolic activation at (slightly) cytotoxic concentrations (Pas84, McG88).
BHT did not induce sex-linked recessive lethal mutations in *D. melanogaster* (Pra74, Maz83, San83).

- Cytogenicity assays. BHT did not induce SCEs or chromosome aberrations in several studies using various cultured Chinese hamster cell lines (ovary, lung, DON, V79) (Bom92, BUA94). However, in the absence of metabolic activation, a dose-related increase in the frequency of chromosome aberrations was observed in cultured human WI-38 embryonic lung cells at concentrations ranging from 2.5 to 250 µg/mL (Bom02, FAO96) and induction of polyploidy in CHO cells at BHT concentrations 5 and 10 µg/mL (Pat87).
- Other assays. BHT gave a positive result in one, modified, *B. subtilus* $rec^{+/-}$ assay while negative results were obtained in 5 other rec-assays performed using standard conditions (Bom02). BHT was negative in the umu test in *S. typhimurium* strain TA1535/pSK1002 at concentrations up to 1320 µg/mL (Hei96) and in the SOS chromotest in *E. coli* strain PQ37 (Bom02, BUA94).
  BHT did not affect the mitotic recombination frequency in *S. cerevisiae* strain D4 or in a host-mediated assay in ICR Swiss mice using *S. cerevisiae* strain D3 at single oral doses of 30, 900, or 1400 mg/kg bw or 5 daily doses of 30, 250, or 500 mg/kg bw (Bom02, BUA94, FAO96).
  In cultured rat hepatocytes, BHT did not induce DNA repair synthesis at concentrations ranging from 0.01 and 10 µg/mL (Wil90a). BHT caused inhibition of replicative DNA synthesis in cultured human HeLa S3 cells, when tested at concentrations ranging from 333 to 1320 µg/mL (Hei96).

- *In vivo* tests:
  Dominant lethal mutations were not induced when male ICR Swiss mice were given a single intraperitoneal injection of 250-2000 mg BHT/kg bw and mated for 8 successive weeks (Eps72). Negative results were also found in

mice treated with BHT via the food for 8 weeks, at a level equivalent to 1500 mg/kg bw/day (She86). In Sprague-Dawley rats, BHT did not induce dominant lethal mutations following single doses of 30, 900, or 1400 mg/kg bw by gavage, but positive results were obtained following repeated doses of 30 or 500, but not 250 mg/kg bw/day, for 5 days (Bru75). Similar positive results were obtained in another study, in which male Sprague-Dawley rats were given 50, 150, or 500 mg/kg bw/day via the diet for 10 weeks, before mating (She86). The outcome of these studies was considered to be positive based on an increased number of dead implants. However, according to current guidelines, results should be evaluated based on comparison of the number of live implants in treated and control animals (and not of death implants). Since no differences in the number of live implants were noted between exposed and control groups, the committee considers these studies to be negative.

BHT did not induce an increased incidence in micronuclei in bone marrow of male and in female mice after a single intraperitoneal injection of 75 mg/kg bw (Pas86) or in female mice, following repeated intraperitoneal doses of 50-1000 mg/kg bw/day, for 5 days (Bru79). In rats, given BHT at single doses of 30, 900, or 1400 mg/kg bw or repeated (5 days) oral doses of 30, 250, or 500 mg/kg bw, no increased incidence of micronuclei in bone marrow cells was observed (Bom02, FAO96). No clastogenic effects were noted in bone marrow of mice (ICR) or rats (Wistar, Sprague-Dawley), given BHT in the diet at doses equivalent to 2000 and 750 mg/kg bw/day, respectively, for 9 months (Bom02).

Changes in migration patterns of DNA in the alkaline comet assay, indicative of DNA damage, were demonstrated in the glandular stomach (at 500 and 1000 mg/kg bw), the colon (at 100, 500, 1000 mg/kg bw), the bladder (at 500, 1000 mg/kg bw), and the brain (at 1000 mg/kg bw) obtained from male ddY mice (n=4/group) 3 hours after single oral doses of 0, 10, 100, 500, or 1000 mg/kg bw. In samples obtained 24 hours after dosing, a ‘positive’ effect was seen in only in the stomach of mice given 1000 mg/kg bw. There were no effects in the lungs, liver, kidneys, and bone marrow (Sas02). Following oral (gavage) administration of 5 mg ^{14}C-BHT to male Wistar rats, relatively small amounts of radioactivity were bound to liver DNA but adduct formation/identification was not addressed (Nak80).

In summary, the majority of tests showed no evidence for BHT to induce gene mutations or other genotoxic effects.

Reproduction toxicity

Following repeated intraperitoneal doses of 50-1000 mg/kg bw/day, for 5 days, sperm abnormalities were observed in mice, 40 days after the last injection of doses of 250 mg/kg bw/day and above (Bru79).

In a 2-generation reproduction study, groups of Wistar albino rats (n=16 females and 2 males/group) were given BHT (purity: >99.5%) via the diet at doses equivalent to 0, 500, 750, or 1000 mg/kg bw/day for 5 weeks, after which time the males and females within each group were mated. Females were maintained on treatment throughout pregnancy and the lactation period. At weaning (21 days after birth), 14 pups from each dose group received control diet for 4 weeks. At the end of the lactation period, body weights of dams were significantly reduced at the 2 higher doses. Absolute and relative liver weights were significantly increased at all dose levels. Livers of dams treated with BHT at 750 and 1000 mg/kg bw/day showed hypertrophy, proliferation of the smooth endoplasmatic reticulum, and proliferation of the bile duct. No microscopic changes were yet seen in livers at day 19 or 20 of gestation. In F1 offspring, there were no differences in the litter or pup weight, number of pups per litter born, or number of pups born dead or dying within 4 days between treated and control rats. No developmental abnormalities were observed in any of the pups. At weaning, pups showed significant, dose-related body and absolute liver weights at all dose levels, which persisted until the end of the experiment, 4 weeks after weaning. The LOAEL for both maternal and reproduction toxicity was 500 mg/kg bw/day (McF97).

In a second experiment, the same authors studied the development of liver changes in the parental (F0) and F1generation of rats, treated under a regimen identical to that producing tumours in the study of Olsen et al. (see ‘Long-term toxicity and carcinogenicity’). Groups of Wistar albino rats (n=50 females and 7 males/group) were given BHT (purity: >99.5%) via the diet at doses equivalent to 0, 25, 100, or 500 mg/kg bw/day for 5 weeks, after which time the males and females within each group were mated. Females were maintained on treatment throughout pregnancy and lactation. In addition, 5 non-pregnant female animals were treated with 0 or 500 mg/kg bw/day, in parallel with lactating dams. Groups of F1 offspring were continued on the same treatment at weaning, except that the high-dose level was reduced to 250 mg/kg bw/day. The study was terminated at week 22 post-weaning (25 weeks after birth). Maternal body weights during pregnancy and lactation were not significantly different in BHT-treated animals compared with untreated controls. No changes were observed in fertility index,

resorption sites per dam, number of fetuses per dam, number of pups per litter born, and pup weight between treated and control rats. Dams sacrificed on days 19 or 20 of gestation did not show significant increased absolute or relative liver weights. At day 21 of lactation, dams and non-lactating rats, treated with 500 mg BHT/kg bw, had significant increased absolute and relative liver weights compared to controls. Microscopic examination of livers of dams revealed centrilobular liver enlargement in rats receiving 100 and 500 mg/kg bw/day and proliferation of smooth endoplasmatic reticulum at 500 mg/kg bw/day. At 100 mg/kg bw/day and above, there was evidence of hyperactivity of the thyroids in some of the animals. Biochemical analyses were conducted on livers of lactating and non-lactating rats treated with 500 mg BHT/kg bw/day and of corresponding controls. BHT-treated rats showed decreased glucose-6-phosphatase (G-6-PP) activity ($p<0.05$ in lactating animals), decreased glutathione (GSH) levels (lactating animals only; $p<0.05$), and increased glutathione-*S*- transferase (GST) activity ($p<0.05$). Of the liver enzymes involved in the metabolism of xenobiotics, the activities of cytochrome P450 (CYP450) ($p<0.05$ in non-lactating animals) and pentoxyresorufin-*O*-dependentylase (PROD) ($p<0.001$) were increased, but the activity of ethoxyresorufin-*O*-deethylase (EROD) was decreased ($p<0.05$ in lactating animals). In F1 offspring of BHT-treated rats, no differences were observed in number of pups dying within 4 days after birth, compared with controls. Dose-related decreased body weights were observed at weaning ($p<0.05$ at 250 mg/kg bw/day), 4 weeks after weaning ($p<0.05$ at 100 and 250 mg/kg bw/day), and 22 weeks after weaning ($p<0.05$, at 250 mg/kg bw/day). At all time points, absolute liver weights remained unchanged in BHT-treated animals compared with controls. Biochemical analyses were conducted on livers from fetuses (delivered on day 19 or 20 of gestation) and from pups at weaning and at 4 and 22 weeks after weaning. A dose-related decrease in G-6-PP activity was observed at all 4 time points, which coincided with the decreases in body weight in BHT-treated animals. GSH concentrations were significantly reduced in pups treated with 250 mg/kg bw/day, 4 and 22 weeks after weaning. GST activities were increased in a dose-related fashion at weaning and 4 and 22 weeks after weaning. Of xenobiotic metabolising enzymes, CYP450 activity was increased ($p<0.05$) at 22 weeks after weaning in pups treated with 250 mg
BHT/kg bw/day. EROD activity was significantly increased at weaning and at 22 post-weaning (at 100 and 200 mg/kg bw/day), and at 4 weeks post-weaning (at 25, 100, and 250 mg/kg bw/day). Benzphetamine *N*-demethylase activity was significantly increased at weaning only and epoxide hydrolase at weaning and at 4 and 22 weeks after weaning, at the high-dose level only. Microscopic

examination showed proliferation of the smooth endoplasmatic reticulum of livers of pups treated with 250 mg/kg bw/day. No other significant changes of the liver were observed. Of the other tissues, mild hyperactivity of the thyroid was observed at doses of 100 and 250 mg/kg bw. The NOAEL for both maternal and reproduction toxicity was 25 mg/kg bw. The maternal NOAEL was based on microscopic changes of the liver and the reproductive NOAEL on decreased body weights, changes in enzyme activities of the liver, and microscopic changes of the thyroid. McFarlane et al. concluded that the only major difference between exposure to BHT of a single generation or over 2 generations is that pups born to dams receiving high doses of BHT (100 mg/kg bw and above) fail to gain weight and develop at the same rate as those born to untreated dams. The most likely explanation is malnutrition of the pups. According to McFarlane et al., this could predispose animals to an increase in incidence of tumours in later life (McF97).

The committee concluded that at 25 mg/kg bw no effects have been observed in rats when exposed *in utero* and post-natally up to 22 weeks of age.

Several other studies have been performed, which are summarised in IARC86 and FAO96. The more recent studies are presented hereunder.

Groups of Wistar (n=40-60/sex/group) received BHT (purity: >99.5%) via the diet at doses equivalent to 0, 25, 100, or 500 mg/kg bw/day, beginning at 13 weeks before mating. Females were maintained on treatment throughout pregnancy and lactation. At 500 mg/kg bw/day, body weights were decreased in male and female rats. There were no differences in gestation index, viability index at birth, and viability index at the end of lactation between BHT-treated and control rats. The litter size, the number of males per litter, and the average birth weight of the pups were significantly lower at 100 and 500 mg/kg bw compared with the controls. During lactation, pup body weights were significantly reduced at all dose levels in a dose-related fashion. The NOAEL for maternal toxicity was 100 mg/kg bw, but a NOAEL for reproduction toxicity could not be established (Ols86).

In another study, groups of male and female Wistar SPF rats (n=46/sex/group) received 0 or 500 mg/kg bw/day of BHT (purity: not given) via the food for 13 weeks before mating and throughout gestation and lactation. Effects of treatment were a decrease in body weight and weight gain before and during gestation. Length of gestation, number of offspring, and birth weight were similar in treated and control animals. Offspring nursed by BHT-treated dams had significantly reduced average body weight and weight gain. Pups exposed to BHT *in utero* showed a relatively slower development than control pups, when fostered with non-treated mothers. Pups exposed to BHT *in utero* or during

lactation showed alterations in auditory and visual function and in locomotive coordination tests, compared with non-BHT-treated pups. Microscopic examination of brain tissue revealed a higher incidence in average number of dead cells (Mey80).

In an earlier experiment, male and female Sprague-Dawley rats were given BHT (purity: not given) via the diet at doses equivalent to 625, 1250, or 2500 mg/kg bw/day, beginning from the week before mating and continuing in females through lactation and weaning. Mortality of pups from dams treated with 1250 or 2500 mg/kg bw/day was significantly higher than controls. Pre-weaning pups from high-dose mothers weighed significantly less than controls at ages 7, 14, and 21 days. Neurobehavioural effects were found in pre-weaning rats at the high-dose level only. Post-weaning males in the 1250 mg/kg bw/day dose group showed an effect on passive avoidance. No effects on basic motor coordination were found in BHT-treated animals. The NOAEL for reproductive and behavioural effects was 625 mg/kg bw/day (Bru78).

In a 3-generation study in mice, groups of Crj:CD-1 mice (n=10/sex/group) received BHT via the diet at levels equivalent to 0, 20, 70, 200, or 615 mg/kg bw/day, starting at 5 weeks of age. At 9 weeks of age, the mice were mated for 5 days. The F1 offspring was weaned at 4 weeks of age, and 10 mice/sex/group were selected for mating at 9 weeks of age. In F1 high-dose pups, a decreased body weight was observed during lactation (day 7 to day 21), but no body weight differences were noted in the BHT-treated F2 pups compared with the controls. No significant effects were noted in reproductive parameters. In the F2 males, one of the neurobehavioural tests (180° turn in the open field trial) was affected during lactation but this effect was not seen in F1 animals or males and not considered of toxicological significance. The NOAELs for reproductive and neurobehavioural effects were 200 and 615 mg/kg bw/day, respectively (Tan93).

7 Existing Guidelines

The current administrative occupational exposure limit (MAC) for BHT in the Netherlands is 10 mg/m^3, 8-hour TWA.

Existing occupational exposure limits for BHT in some European countries and in the USA are summarised in Annex II.

8 Assessment of health hazard

Workers can be exposed to BHT through inhalation of dust or aerosol or by direct skin contact when handling the compound. No data is available on the

percentage uptake of the compound through the lungs. The dermal absorption of BHT in a lipophilic vehicle in guinea pigs was maximally 3% of the applied dose within 24 hours after application. Following oral intake, absorption was at least 75% of the dose in humans, 80-90% in rats, 85% in guinea pigs, and close to 100% in mice. Following repeated application, BHT will accumulate in the fat and, to a lesser extent, in the liver. The half-life of elimination from these tissues was 5-15 days in the rat. Metabolism of BHT occurs via 3 main pathways, i.e., oxidation of the 4-methyl group, oxidation of one or both *t*-butyl moieties, and ring oxidation, resulting in a range of breakdown products. The latter pathway involves formation of the reactive metabolite butylhydroxytoluene quinone methide, which may bind covalently to biomacromolecules in tissues. This pathway was demonstrated in rats, but not in humans, where only products of oxidation and conjugation of alkyl substituents were identified as urinary components. Following a single oral dose, most of the metabolites are excreted in the urine and the faeces within 24 hours after application. The committee concludes that repeated doses of BHT lead to accumulation in fatty tissues, which are cleared within 1-2 weeks without BHT exposure.

Case studies in humans showed that BHT is mildly irritating to the skin and in some cases a skin sensitiser. Incidental oral intake of estimated doses of 4 and 80 g of BHT did not cause fatalities.

In experimental animals, BHT was slightly irritating to the skin and the eyes of rabbits, but no skin sensitisation was demonstrated in guinea pigs. However, BHT vapour was irritating to the lungs of mice, at airborne levels between 17 and 47 mg/m^3 (1.9 and 5.1 ppm). The estimated RD_{50} for sensory irritation was 33 mg/m^3 (3.6 ppm). No acute lethal inhalation or dermal toxicity studies with BHT have been reported. Based on the results of acute oral toxicity studies in rats and mice, the committee considers the compound not to present an acute health hazard following oral intake. In rats, symptoms of poisoning following acute high oral doses were haemorrhages, which are possibly due to inhibition of vitamin K-dependent blood coagulation factors II, VII, IX, and X by the metabolite BHT-quinone methide. Considerable differences were noted in sensitivity between different strains of mice to the acute effects of BHT following intraperitoneal injection. According to the committee, this difference in sensitivity is due to genetic differences in BHT metabolism.

Short-term dermal exposure to BHT caused lung damage in mice at levels >200 mg/kg bw/day. At oral doses >25 mg/kg bw/day, decreased body weights and effects on the liver have been reported in rats and mice as well as renal injury in mice. In monkeys, rats, and mice, induction of hepatic enzymes involved in the metabolism of xenobiotics was measured, accompanied in rats and mice by

proliferation of the smooth endoplasmatic reticulum and liver enlargement, leading to increased liver weights. Oral administration of doses of BHT of 125 mg/kg bw/day for 28 days to Sprague-Dawley rats, supplied with recommended dietary amounts of vitamin K, did not cause effects on blood coagulation. Experiments in male and female rats of 3 different strains, male mice of 5 different strains, male rabbits, and male dogs suggested that these effects were species and strain dependent.

Non-neoplastic effects of long-term exposure to BHT in rats, at doses >75 mg/kg bw/day, were reduced body weight and increased absolute and relative liver weights. In mice, effects were decreased body weights and liver injury at doses of 450 mg/kg bw and above.

In some genotoxicity tests, positive results were obtained, amongst others at (slightly) cytotoxic concentrations in the presence of a metabolic activating system in mutation assays with cultured mammalian cells. However, since BHT was negative in most *in vitro* and *in vivo* genotoxicity assays, the committee considers BHT not to be a mutagenic/genotoxic compound.

With regard to carcinogenicity, BHT showed an increased incidence of pulmonary tumours in female mice at the lower (450 mg/kg bw/day), but not at the higher (900 mg/kg bw/day) dose level. In 3 other studies, there was no difference in tumour incidence among treated and control groups. In one study in rats, an increased incidence in pituitary adenomas was seen in low-dose (125 mg/kg bw/day), but not in high-dose (500 mg/kg bw/day) animals. In another rat study, hepatocarcinomas were observed following lifetime exposure to 250 mg BHT/kg bw/day of a group of rats from dams treated with BHT before mating and throughout pregnancy and lactation. The committee is of the opinion that it is difficult to draw conclusions about the observed incidence of liver carcinomas in the treated group, because of the large differences in survival between treatment and control groups. Two other carcinogenicity studies in rats did not show differences in tumour incidence among treated and control groups. In view of the lack of evidence for mutagenicity or genotoxicity of BHT, the committee considers that the carcinogenicity in mice and rats, observed in some of the above long-term experiments, is not through genotoxic mechanisms. The committee agrees with the conclusion of IARC, that ‘the carcinogenicity of BHT to humans could not be evaluated’.

In reproductive toxicity studies in rats, the most sensitive treatment-related effects in offspring were a decrease in body weight and induction of xenobiotic-metabolising hepatic enzymes at doses of 100 mg/kg bw/day and above, beginning at the lactation period and continuing for at least 22 weeks post-weaning. Treatment-related decreased body weights generally occurred at a

lower level in offspring than in dams. Fetotoxicity was observed in one study only, at 1250 mg/kg bw and above.

Based on the above data, the committee concludes that decreases in body weight in BHT-treated offspring and hepatic enzyme induction are the most sensitive toxic effects in animal studies. These effects have not been demonstrated in short- and long-term studies at oral doses of 25 mg/kg bw/day and below, and in the key 2-generation reproductive study of Mc Farlane et al. (McF97). The committee takes the NOAEL of 25 mg/kg bw/day from this study as a starting point in establishing a health-based recommended occupational exposure limit (HBROEL). Since workers are exposed for 5 days a week, this NOAEL from a continuous study (i.e., 7 days a week) is adjusted by multiplying with a factor of 7/5 resulting in a no-adverse-effect level (NAEL) of 35 mg/kg bw. For the extrapolation to a HBROEL, a factor of 4 for allometric scaling from rats to humans, based on caloric demand, and an overall factor of 18, covering inter- and intraspecies variation and the differences between experimental conditions and the exposure pattern of the workers, are applied, resulting in a NAEL for humans of 0.5 mg/kg bw/day. Assuming a 70-kg worker inhales 10 m^3 of air during an 8-hour working day and a retention of 100%, and applying the preferred value approach, a HBROEL of 5 mg/m^3 is recommended for BHT.

The committee recommends a health-based occupational exposure limit of 5 mg/m^3 for 2,6-di-*tert*-butyl-*p*-cresol, as inhalable dust, as an 8-hour time-weighted average (TWA).

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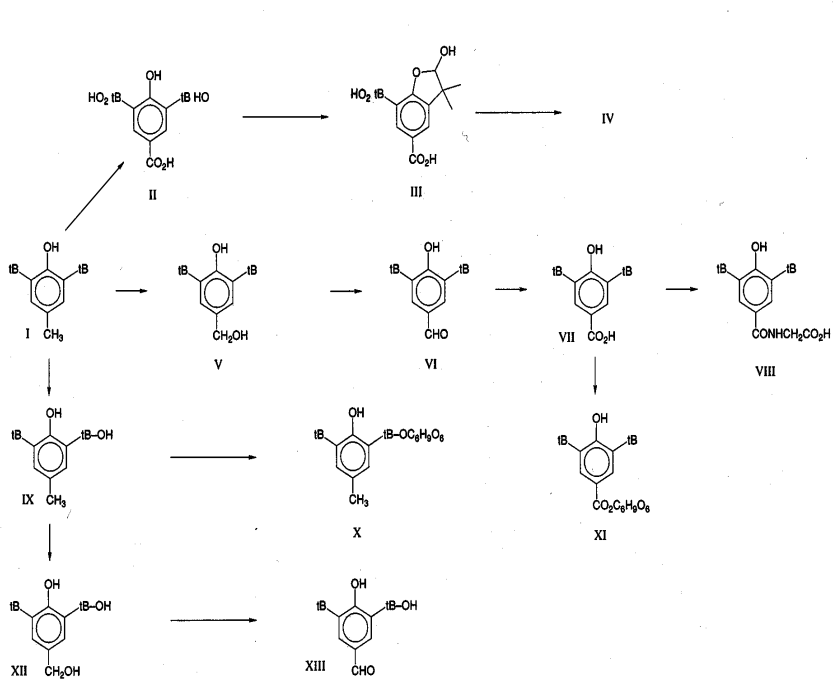
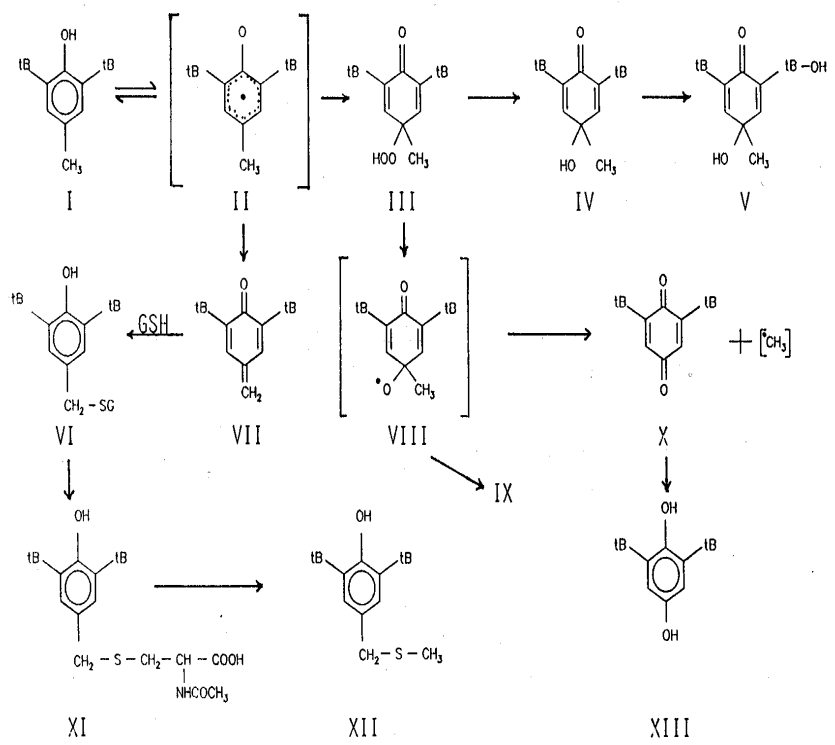
Annex I



I 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT)
II 4-carboxy-2-(1-carboxy-1-methylethyl)-6-(1-formyl-1-methylethyl)phenol
III 5-arboxy-7-(1-carboxy-1-methylethyl)-3,3-dimethyl-2-hydroxy-2,3-dihydroxybenzofuran
IV glucuronide of III
V 2,6-di-*tert*-butyl-4-hydroxymethylphenol (BHT-OH, BHT-alcohol)
VI 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO, BHT-aldehyde)
VII 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (BHT-COOH, BHT-acid)
VIII 3,5-di-*tert*-butyl-4-hydroxymethylphenol-glycin conjugate
IX α-hydroxy-2,6-di-*tert*-butyl-*p*-cresol (α-hydroxy-BHT)
X α-hydroxy-2,6-di-*tert*-butyl-4-hydroxymethylphenol glucuronide
XI 3,5-di-*tert*-butyl-4-hydroxybenzoic acid ester-glucuronide
XII α-hydroxy-2,6-di-*tert*-butyl-4-hydroxymethylphenol
XIII α-hydroxy-2,6-di-*tert*-butyl-4-hydroxybenzaldehyde

Figure 1a Metabolic pathways of BHT: oxidation and conjugation of alkyl substituents (from BUA94; see also Wit89).



I 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT)
II phenoxy radical
III 2,6-di-*tert*-butyl-4-hydroperoxy-4-methylcyclohexa-2,5-diene-1-one (BHT-OOH, hydroxyperoxy-BHT)
IV 2,6-di-*tert*-butyl-4-hydroxy-4-methylcyclohexa-2,5-diene-1-one
V α-hydroxy-2,6-di-*tert*-butyl-4-hydroxy-4-methylcyclohexa-2,5-diene-1-one
VI S-(3,5-di-*tert*-butyl-4-hydroxybenzyl)glutathione (BHT-glutathione)
VII 2,6-di-tert-butyl-4-methylene-cyclohexa-2,5-diene-1-one (BHT-quinone methide)
VIII quinoxy radical
IX rearrangment products
X 2,6-di-*tert*-butyl-p-benzoquinone
XI *S*-(3,5-di-tert-butyl-4-hydroxybenzyl)-N-acetylcysteine (BHT mercapturic acid)
XII 2,6-di-*tert*-butyl-4-(methylthio)methylphenol
XIII 2,6-di-*tert*-butylhydroquinone

Figure 1b Metabolic pathways of BHT: ring oxidation (from BUA94; see also Wit89).

Annex II

Occupational exposure limits for butylated hydroxytoluene in various countries.

country - organisation	occupational exposure limit		time-weighted average	type of exposure limit	note[a]	reference[b]
	ppm	mg/m^3				
the Netherlands						
- Ministry of Social Affairs and Employment	-	10				SZW03
Germany						
- AGS	-	10[c]	8 h	administrative		TRG00
- DFG MAK-Kommission	-	-[d]				DFG03
Great Britain						
- HSE	-	10	8 h	OES		HSE02
Sweden	-	-				Swe00
Denmark	-	10	8 h			Arb02
USA						
- ACGIH	-	2[c]	8 h	TLV	A4[e]	ACG03b
- OSHA	-	-	-			ACG03a
- NIOSH	-	10	10 h	REL		ACG03a
European Union						
- SCOEL	-	-				EC04

[a] S = skin notation, which means that skin absorption may contribute considerably to body burden; sens = substance can cause sensitisation.

[b] Reference to the most recent official publication of occupational exposure limits.

[c] Inhalable fraction of vapour plus aerosol.

[d] Listed among compounds for which studies of the effects in man or experimental animals have yielded insufficient information for the establishment of MAK values.

[e] Classified in carcinogenicity category A4, i.e., not classifiable as a human carcinogen: agents which cause concern that they could be carcinogenic for humans but which cannot be assessed conclusively because of a lack of data. *In vitro* or animal studies do not provide indications of carcinogenicity which are sufficient to classify the agent into one of the other categories.