**SCIENTIFIC OPINION**

# Scientific Opinion on the re-evaluation of butylated hydroxytoluene BHT (E 321) as a food additive[1]

**EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)[2, 3]**

European Food Safety Authority (EFSA), Parma, Italy

## ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food (ANS) delivers an opinion re-evaluating the safety of butylated hydroxytoluene (BHT) (E 321). BHT is an authorised synthetic antioxidant that was previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the latest in 1996 and the EU Scientific Committee for Food (SCF) in 1987. The SCF established an ADI of 0-0.05 mg/kg bw/day based on thyroid, reproduction and haematological effects in the rat. JECFA allocated an ADI of 0-0.3 mg/kg bw/day for BHT based on effects in the reproduction segments and hepatic enzyme induction seen in two separate 2-generation studies in rats. The Panel concluded that BHT is not of concern with respect to genotoxicity and that any carcinogenicity would be thresholded. After the last SCF evaluation, two new 2-generation studies have been reported which were the basis for the ADI set by JECFA. Both studies revealed a NOAEL of 25 mg/kg bw/day. Overall, the Panel concluded that the present database gives reason to revise the ADI of 0.05 mg/kg bw/day. Based on the NOAEL of 25 mg/kg bw/day and an uncertainty factor of 100, the Panel derived an ADI of 0.25 mg/kg bw/day. Since the NOAEL of 25 mg/kg bw/day is below the BMDL10 value of 247 mg/kg bw/day derived from the data for the incidence of hepatocellular carcinomas in male rats, the Panel concluded that this NOAEL also covers the hepatocellular carcinomas observed in the long-term studies with BHT. Exposure of adults to BHT is unlikely to exceed the newly derived ADI at the mean and at the 95th percentile. For exposure of children to BHT from its use as food additive, the Panel noted that it is also unlikely that this ADI is exceeded at the mean, but is exceeded for some European countries (Finland, The Netherlands) at the 95th percentile.



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**KEY WORDS**

BHT, butylated hydroxytoluene, 2,6-di-tert-butyl-p-cresol, butylhydroxytoluene, 4-methyl-2,6-ditertiarybutylphenol, E 321, CAS 128-37-0, food antioxidant.

**SUMMARY**

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion on the re-evaluation of butylated hydroxytoluene (BHT) (E 321) as a food additive.

BHT (E 321) is a synthetic antioxidant authorised as a food additive in the EU that was previously evaluated by the EU Scientific Committee for Food (SCF) in 1987 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) several times, the latest in 1996.

The SCF established an ADI of 0-0.05 mg/kg bw/day based on thyroid, reproduction and haematological effects in the rat. At its last evaluation JECFA allocated an ADI of 0-0.3 mg/kg bw/day for BHT, based on effects in the reproduction segments and hepatic enzyme induction, seen in two separate 2-generation studies in rats. These studies were probably not available at the time of the SCF evaluation.

Specifications have been defined in the EU legislation Directive 2008/48/EC and by JECFA (JECFA, 2006). The purity is specified to be not less than 99%.

Absorption, distribution, metabolism and excretion of BHT have been studied in mice, rats, rabbits, chickens, monkeys and humans. Overall, these studies show that BHT is rapidly absorbed from the gastrointestinal tract. Upon absorption BHT is distributed to the liver and body fat, while excretion is mainly via urine and faeces. The metabolism of BHT is complex and there may be important species differences. It is not known, for example, whether humans are capable of forming the quinone methides, metabolites that were found in rats and mice. In addition, biliary excretion seems not to be as significant in man as it is in rats, rabbits and dogs.

The acute toxicity of BHT is low with oral $LD_{50}$ values of 1700-1970 mg BHT/kg bw in rats, 2100-3200 mg BHT/kg bw in rabbits, 10 700 mg BHT/kg bw in guinea pigs, 940-2100 mg BHT/kg bw in cats, and 2000 mg BHT/kg bw in mice.

In general, the genotoxicity studies on BHT indicate a lack of potential for BHT to induce point mutations, chromosomal aberrations, or to interact with or damage DNA. The Panel recognised that positive genotoxicity results obtained with BHT in vitro may be due to pro-oxidative chemistry giving rise to formation of quinones and reactive oxygen species and that such a mechanism of genotoxicity is generally considered to be subject to a threshold.

The Panel noted that the SCF established an ADI of 0-0.05 mg/kg bw/day based on thyroid, reproduction and haematological effects in the rat. However, a NOAEL of 25 mg BHT/kg bw/day was derived from a study in rats where electron microscopy of the thyroid glands of rats exposed to 500 mg BHT/kg bw/day for 28 days showed an increase in the number of follicle cells.

The Panel noted the discrepancy between the ADIs allocated by the SCF and JECFA. In 1987 the SCF reviewed all available studies on BHT, among them metabolic data from several species including man, mutagenicity studies, carcinogenicity studies in rats and mice, special studies on the thyroid, blood, and post-natal development and behaviour. Taking all these effects into account, the SCF considered that the likely NOAEL for BHT is approximately 100 mg/kg in the diet, equivalent to an

intake of about 5 mg/kg bw/day. In the view of the nature of the effects, a safety margin of 100-fold was considered appropriate to establish an ADI of 0-0.05 mg/kg bw based on thyroid, reproduction and haematological effects in the rat. The studies used by JECFA to define the ADI were published after the last SCF evaluation and these studies have been included in the present evaluation.

After the last SCF evaluation, two new 2-generation studies have been reported. One study has been published by Olsen et al. in 1986. The other study is an unpublished report by Price in 1994 that was included in the JECFA evaluation published in 1996 and also submitted to EFSA after a public call for data.

The Panel considered that the effects of BHT on tumour formation reported in the Olsen et al. study in 1986 are subject to a threshold since the genotoxicity studies generally indicate a lack of potential for BHT to induce point mutations, chromosomal aberrations, or to interact with or damage DNA. The BMD analysis performed by the Panel on the incidence of hepatocellular carcinoma in male rats induced by BHT as reported by Olsen et al. in 1986 gave a $BMDL_{10}$ of 247 mg/kg bw/day.

Both two new studies indicated a NOAEL of 25 mg/kg bw/day. In the Olsen et al. study this NOAEL of 25 mg/kg bw/day is based on effects on litter size, sex ratio and pup body weight gain during the lactation period in the reproduction segment of the study. The Panel agreed with the NOAEL of 25 mg/kg bw/day derived by JECFA from the study reported by Price in 1994.

Overall, the Panel concluded that the present database does give reason to revise the ADI of 0-0.05 mg/kg bw/day set by the SCF.

Based on the NOAEL of 25 mg/kg bw/day, derived from both new 2-generation studies, and an uncertainty factor of 100 the Panel established an ADI for BHT of 0.25 mg/kg bw.

The NOAEL of 25 mg/kg bw/day for the reproductive effects is below the $BMDL_{10}$ value of 247 mg/kg bw/day derived by the Panel from the data of Olsen et al. for the incidence of hepatocellular carcinoma in male rats. The Panel concluded that this NOAEL of 25 mg/kg bw/day also covers the hepatocellular carcinomas observed in the long term studies with BHT.

Using a worst-case scenario of combined exposure to BHT from the food categories where use as a food additive is authorised, the Panel estimated potential exposure for adults to be on average 0.01-0.03 mg/kg bw/day and 0.03-0.17 mg/kg bw/day at the 95$^{th}$ percentile. For children, the Panel estimated potential exposure in the range of 0.01-0.09 mg/kg bw/day at the mean and in the range of 0.05-0.30 mg/kg bw/day at the 95$^{th}$ percentile, The Panel noted that at the mean exposure of adults to BHT is unlikely to exceed the newly derived ADI of 0.25 mg/kg bw/day and at the 95$^{th}$ percentile. For exposure of children to BHT from its use as food additive, the Panel noted that it is also unlikely that this ADI is exceeded at the mean, but is exceeded for some European countries (Finland, The Netherlands) at the 95$^{th}$ percentile.

The Panel noted that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$5 mg/kg.

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008[4] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under Regulation (EU) No 257/2010[5]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[6] of 2001. The report "Food additives in Europe 2000[7]" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010 the 2003 Terms of Reference are replaced by those below.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedure and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

[4] OJ L 354, 31.12.2008, p. 16.
[5] OJ L 80, 26.03.2010, p19
[6] COM(2001) 542 final.
[7] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers. TemaNord 2002:560.

## ASSESSMENT

### 1. Introduction

The present opinion deals with the re-evaluation of the safety of butylated hydroxytoluene (BHT) (E 321) when used as a food additive.

BHT (E 321) is a synthetic antioxidant authorised as a food additive in the EU that was previously evaluated by the EU Scientific Committee for Food (SCF) in 1987 and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) several times, the latest in 1996.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data.

### 2. Technical data

#### 2.1. Identity of the substance

Butylated hydroxytoluene (BHT) (E 321) is a synthetic antioxidant with the formula $C_{15}H_{24}O$. It has a molecular weight of 220.36 g/mol; the CAS Registry Number is 128-37-0 and the EINECS number is 204-881-4. The chemical name is 4-methyl-2,6-ditertiarybutylphenol and the structural formula is presented in Figure 1.

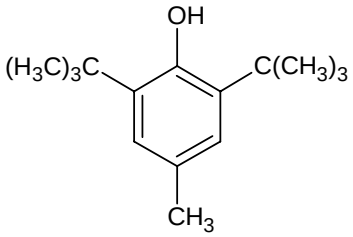


**Figure 1:** Structural formula of BHT

The most commonly used synonyms are butylated hydroxytoluene, butylhydroxytoluene, BHT and 2,6-ditertiary-butyl-p-cresol.

BHT is a white, crystalline solid which is odourless or has a faint aromatic odour. BHT is insoluble in water, and freely soluble in ethanol and fatty oils. It has a melting point of 70 °C and an octanol/water partition coefficient (log $P_{ow}$) of 5.1 (IPCS, 1999; JECFA, 1967; TemaNord, 2002).

#### 2.2. Specifications

Specifications for BHT have been defined in Commission Directive 2008/84/EC[8] on purity criteria and by JECFA (JECFA, 2006) (Table 1).

[8] Commission Directive 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners. OJ L 253, 20.9.2008, p.1.

**Table 1:** Specifications for BHT according to Commission Directive 2008/84/EC and to JECFA (2006).

| | Commission Directive 2008/84/EC | JECFA (2006) |
|---|---|---|
| **Assay** | ≥ 99% | ≥ 99.0% |
| **Solubility** | Insoluble in water and propane-1,2-diol; freely soluble in ethanol | Insoluble in water and propane-1,2-diol; freely soluble in ethanol |
| **Melting point/range** | 70 °C | 69 – 72 °C |
| **Spectrophotometry** | The absorption in the range 230 to 320 nm of a 2 cm layer of a 1:100000 solution in dehydrated ethanol exhibits a maximum only at 278 nm.<br><br>Specific absorption $E^{1\%}_{1cm}$ (278 nm) in ethanol: ≥ 81 and ≤ 88. | The absorption in the range 230 to 320 nm of a 2 cm layer of a 1:100000 solution in dehydrated ethanol exhibits a maximum only at 278 nm. |
| **Solidification** | - | ≥ 69.2 °C |
| **Sulphated ash** | ≤ 0.005% | ≤ 0.005% |
| **Lead** | ≤ 5 mg/kg | ≤ 2 mg/kg |
| **Phenolic impurities** | ≤ 0.5% | ≤ 0.5% |
| **Arsenic** | ≤ 3 mg/kg | - |
| **Mercury** | ≤ 1 mg/kg | - |
| **Heavy metals (as Pb)** | ≤ 10 mg/kg | - |

The Panel noted that the JECFA specification for lead is ≤ 2 mg/kg whereas the EC specification is ≤ 5 mg/kg. The Panel also noted that in the EC specifications arsenic and mercury are allowed at levels up to 3 mg/kg and 1 mg/kg, respectively, whereas no specifications for arsenic and mercury have been provided by JECFA.

The Panel noted that the limit test for heavy metals (expressed as lead) is considered obsolete and is being replaced with limits for individual metals of concern.

### 2.3. Manufacturing process

According to the Hazardous Substances Data Bank HSDB (2010), Budavari (1996) and information available to EFSA, BHT is prepared in a multistep process by the reaction of p-cresol (4-methylphenol) with high purity isobutylene (2-methylpropene) using an acid catalyst. Upon neutralization by addition of sodium carbonate, crystallization with isopropanol, filtration and washing with isopropanol, the substance is dried and sieved to obtain the final product.

### 2.4. Methods of analysis in food

According to JECFA (1990) specifications BHT can be detected by gas chromatography with a flame ionization detector (FID). A number of other methods have been found in the literature including high-performance liquid chromatography (HPLC), gas-chromatography coupled with mass spectrometry (GC-MS) and a direct Fourier transform infrared (FTIR) spectroscopic method (Yankah et al., 1998; Fries and Puttmann, 2002, 2004; Tombesi and Freije, 2002; Witter, 2005; Ammawath et al., 2006).

### 2.5. Reaction and fate in food

BHT has been shown to degrade over time when heated during deep fat frying (Warner et al., 1986). The major oxidation product of BHT is HBHT (2,6-di-tert-butyl-4-hydroperoxy-4-methylcyclohexa-2,5-dien-1-one; CAS Registry Number 6485-57-0) (Karasch and Joshi, 1957). A second route of decomposition gives rise to formation of intermediate alkoxy radicals which in turn react to form predominantly MHCD (2,6-di-tert-butyl-4-hydroxy-4-methylcyclohexa-2,5-dione; CAS Registry Number 10396-80-2) (Warner, 1986). BHT is sensitive to visible-light photo-irradiation and traces of heavy metals (Criado et al., 2007; Ph. Eur. Commentary, 2004).

### 2.6. Case of need and proposed uses

Authorised use levels have been defined in Council Directive No 95/2/EC[9] on food additives.

BHT is a synthetic antioxidant authorised for use in fats and oils, only for the professional manufacture of heat-treated food, in frying oil and frying fat (excluding olive pomace oil) and in lard, fish oil, beef, poultry and sheep fat. It is permitted alone or in combination with other antioxidants such as gallates, tert-butylhydroquinone (TBHQ) and butylated hydroxyanisole (BHA) in amounts up to 100 mg/kg expressed as fat. In addition, BHT is permitted in chewing gum alone or in combination with the aforementioned antioxidants at a maximum level of 400 mg/kg chewing gum (Directive No 95/2/EC).

**Table 2:** Maximum Permitted Levels (MPLs) of BHT in foodstuffs according to Directive No 95/2/EC and maximum reported use levels of BHT in foodstuffs used for the refined exposure assessment

| **Foodstuffs** | **Maximum Permitted Level** (mg/kg) | **Maximum reported use level (mg/kg)** |
|---|---|---|
| Fats and oils for the professional manufacture of heat-treated foodstuffs | 100 (a) | 100 (b) |
| Frying oil and frying fat, excluding olive pomace oil | 100 (a) | * |
| Lard; fish oil; beef, poultry and sheep fat | 100 (a) | 100 (b) |
| Chewing gum | 400 (a) | * |
| Food supplements as defined in Directive 2002/46/EC[10] | 400 (a) | * |

(a): When combinations of the antioxidants gallates, TBHQ, BHA and BHT are used, the individual levels must be reduced proportionally.
(b) Maximum reported use levels from the industry
* Industry reported that no data were received from members

#### 2.6.1. Reported use levels for BHT

No data on the actual levels of BHT in foods have been found during literature searches in the databases ToxNet, PubMed and CAPlus, or on the web pages of the Food Standards Agency (FSA) of Great Britain.

The Danish Veterinary and Food Administration has reported a project on monitoring and control of food additives in which BHT levels were analysed in 122 samples of emulsified and non-emulsified

[9] European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. OJ L 61, 18.3.1995, p. 1.
[10] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51.

sauces (dressings, ketchup etc.) and fruit- and vegetable preparations (chutney, tomato paste etc.). BHT was not identified in any of these samples (Jensen, 2006).

When scrutinizing ingredient lists on various chewing gums on the Danish market, BHT is not mentioned in the list of ingredients on every brand. Hence, it can be deduced that chewing gum may be manufactured without the use of BHT. In a survey by Bemrah et al. (2008) 1 out of 28 chewing gum brands was found to contain 200 mg BHT/kg, whereas the remainder contained no BHT.

Additional information on reported use levels of use for BHT was made available to the Panel by FoodDrinkEurope. For the food categories "fats and oils for the professional manufacture of heat-treated foodstuffs" and for "lard, fish oil, beef, poultry and sheep fat" the data made available are listed in Table 2. For other food categories where the use of BHT is authorised, FoodDrinkEurope either reported that no data were received from their membership or that these categories are not representatively covered by FoodDrinkEurope's membership. BHT is also used in food contact materials with a specific migration limit of 3 mg/kg food (Commission Regulation 10/2011[11]).

### 2.7. Information on existing authorisations and evaluations

In 1987 the SCF reviewed all available studies on BHT, including mutagenicity studies, carcinogenicity studies in rats and mice, special studies on the thyroid, blood, post-natal development and behaviour and metabolic data from several species (SCF, 1989). The SCF concluded that the 5% reduction in pup weight seen in rats dosed in utero with 25 mg/kg bw/day indicates that the No Observed Adverse Effect Level (NOAEL) for this effect is even lower. Taking into account the thyroid, reproduction and haematological effects in the rat with a NOAEL of 100 mg/kg in the diet, equivalent to about 5 mg/kg bw/day, and an uncertainty factor of 100, the SCF established an ADI of 0-0.05 mg/kg bw/day (SCF, 1989).

BHT was previously evaluated by JECFA at the sixth, eighth, ninth, seventeenth, twentieth, twenty-fourth, twenty-seventh, thirtieth, and thirty-seventh meetings. At the thirty-seventh meeting, the temporary ADI of 0 - 0.125 mg/kg bw, established at the thirtieth meeting, was extended, pending the results of a study designed to elucidate the role of hepatic changes in the development of hepatic carcinomas observed in Wistar rats following in utero and lifetime exposure to BHT (JECFA, 1996).

In view of the probable involvement of hepatic enzyme induction in the development of the hepatocellular damage associated with exposure to repeated doses of BHT, JECFA (1996) stated that a well-defined threshold was demonstrated at 100 mg/kg bw/day in the long-term study reviewed for the first time at this meeting, giving a NOAEL of 25 mg/kg bw/day. Effects observed in the reproduction segments of the in utero/lifetime exposure studies were also taken into account in the derivation of this NOAEL. The Committee used an uncertainty factor of 100 to allocate an ADI of 0-0.3 mg/kg bw/day for BHT (JECFA, 1996). The evaluation was mainly based on the studies of Olsen et al. (1986) and Price (1994). In addition, JECFA (1996) took into consideration new data relating to the previously noted effects of BHT on the lung, liver, kidney, clotting mechanisms and promotion/inhibition of carcinogenesis, new long-term and reproductive toxicity studies, genotoxicity assays and human observations.

The International Agency for Research on Cancer (IARC) evaluated BHT (1987) and classified it in group 3, since no evaluation of the carcinogenicity of BHT in humans could be made, and there was limited evidence for the carcinogenicity in experimental animals.

The OECD evaluation (OECD, 2002) was in line with the JECFA evaluation (JECFA, 1996). The OECD report indicated that upon chronic oral exposure of rats, liver and thyroid are the main targets

[11] Commission Regulation No 10/2011 of 14 January 2011 on plastic materials intended to come into contact with food. OJ L 12, 15.01.2011, p 12.

and that 25 mg/kg bw/day BHT can be considered as NOAEL for chronic exposure. The OECD report also stated that the haemorrhagic effects of high repeated doses of BHT seen in certain strains of mice and rats, but not in other species, may be related to its ability to interact with prothrombin and vitamin K. It was also concluded that BHT is not a genotoxic carcinogen, but that it cannot be excluded that high and chronic doses of BHT may result in persistent cell proliferation, which is known as a possible mechanism of non-genotoxic carcinogens. It was stated that for the possible carcinogenic and tumour promoting effect of BHT, a threshold level of 100 mg/kg bw/day can be assumed. The NOAEL for effects on reproduction, resulting in lower numbers of litters of ten or more pups at birth was 25 mg/kg bw/day.

## 2.8. Exposure

The Panel agreed to follow the principles of the stepwise approach, which were used in the report of the scientific cooperation (SCOOP) Task 4.2 (EC, 1997), to estimate food additives' intakes. For each successive Tier, this involved a further refinement of intake estimates. The approach progresses from the conservative estimates that form the first Tier of screening, to more realistic estimates that form the Second and Third Tiers.

### 2.8.1. Refined estimates

Refined exposure estimates are usually performed for Tier 2 using national consumption data and maximum permitted use levels presented in Table 2, and for Tier 3, using the maximum reported use levels for children and the adult population. As data made available on reported use levels of BHT were equal to those of maximum permitted use levels, Tier 3 estimates are also equal to Tier 2 estimates.

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been built from existing national information on food consumption at a detailed level. Competent authorities in the European countries provided EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country. This included food consumption data from infants (2 surveys from 2 countries), toddlers (8 surveys from 8 countries), children (17 surveys from 14 countries), adolescents (14 surveys from 12 countries), adults (21 surveys from 20 countries) elderly (9 surveys from 9 countries) and very elderly (8 surveys from 8 countries) from a total of 32 different dietary surveys carried out in 22 different European countries. Surveys on children (from 13 different European countries) were mainly obtained through the Article 36 project "Individual food consumption data and exposure assessment studies for children" (acronym EXPOCHI) (Huybrechts et al., 2010).

Overall, the food consumption data gathered at EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, consumption data were collected by using different methodologies and thus they are not suitable for direct country-to-country comparison. However, consumption data were collected by different methodologies and thus direct country-to-country comparison should be made with caution.

The Panel considered that only chronic dietary exposure to BHT needs to be assessed. Therefore, as suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011b) dietary surveys with only one day per subject were not considered for the calculation of exposure to BHT, as they are not adequate to assess repeated dietary exposure. Similarly, subjects who participated only one day in the dietary studies where the protocol prescribed more reporting days per individual were excluded.

The Panel estimated exposure for the population groups for which sufficiently reliable consumption data were available: children, adolescents, and adults.

Consumption records were codified according to the FoodEx classification system (EFSA, 2011a). Nomenclature from FoodEx classification system has been linked to the Food Classification System as presented in the Commission Regulation (EU) N° 1129/2011[12], part D, to perform exposure estimates.

Further details on how the Comprehensive Database is used are published in the Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

Exposure to BHT from its use as food additive has been calculated by using data on reported use levels as listed in Table 2 combined with national consumption data for the four population groups toddlers, other children, adolescents, and adults. Since the food categories, where BHT is authorised are not directly covered in the Comprehensive Database, it was assumed that the food category for which BHT is authorised can be found in fine bakery wares, in snacks (dry, savoury potato, cereal or starch-based snack products, extruded or expanded savoury snack products, other savoury snack products and savoury peanuts, nuts or hazelnuts), and in liquid and solid food supplements. It was also assumed that the fat content of these food categories would be 25% and that this amount of fat would contain BHT added at the maximum reported use level.

The Panel noted that its estimates should be considered as being conservative as it is assumed that all processed foods contain 25% of fat with BHT added at the maximum reported use levels.

Data from the Comprehensive Database were used by the Panel to calculate both mean and high level exposure to BHT after recoding of the food categories in the Comprehensive Database according to the EU food nomenclature (FCS) and using (1) the MPLs listed in Table 2 and (2) the levels listed in Table 2 (refined exposure assessment). As these values are equal, therefore exposure estimates at Tier 2 and Tier 3 are also equal. High level exposure ($95^{th}$ percentile of consumers only) was calculated adding the exposure at the $95^{th}$ percentile resulting from the consumption of the main contributing food group to the mean exposure resulting from the consumption of all other food groups. This is based on the assumption that an individual might be a high level consumer of one food category and would be an average consumer of the others. This approach has been tested several times by the Panel in re-evaluation of food colours and has shown reasonable correlation with high level total intakes when using the raw food individual consumption data. Therefore, this approach was preferred for the calculations based on the maximum permitted level/maximum reported use levels in order to avoid excessively conservative estimates. High level exposure was only estimated for those foods and population groups were the sample size was sufficiently large to allow calculation of the $95^{th}$ percentile.

[12] Commission Regulation (EU) No 1129/2011of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additive. OJ L 295 12.11.2011, p 1-177.

Table 3 summarises the estimated exposure to BHT from its use as food additive of all four population groups.

**Table 3:** Summary of anticipated exposure to BHT from its use as food additive using maximum reported use levels for four population groups

| | **Children (3-9 years)** mg/kg bw/day | **Adolescents (10-17 years)** mg/kg bw/day | **Adults (18-64 years)** mg/kg bw/day |
|---|---|---|---|
| **Tier 2/Tier 3**. Estimated exposure using maximum reported use levels | | | |
| • Mean exposure | 0.007-0.087 | 0.005-0.043 | 0.003-0.022 |
| • Exposure 95th percentile | 0.04-0.296 | 0.029-0.125 | 0.017-0.161 |

An exposure to BHT from the consumption of chewing gum using the MPL for this food category of 400 mg/kg would require a daily intake of 7.5 g of chewing gum to reach a level of 0.05 mg BHT/kg bw/day. Unfortunately, very limited data were available for the consumption of this food category in the Comprehensive Database since only few consumers in some countries reported data on chewing gum consumption. It also has to be considered that not all BHT present in chewing gum will be ingested. It was concluded that 80-99% of BHT in chewing gum is not ingested (Nunn, 1991), but ingestion may be higher given that higher levels of extraction of up to 50% were also found in one study (Sato and Kawamura, 1972). Assuming a 10% extraction and subsequent ingestion of BHT from chewing gum and selecting only those surveys with a sufficiently high number of consumers, the average exposure to BHT from chewing gum consumption is in the range of 0-0.003 mg/kg bw/day for children, 0-0.002 mg/kg bw/day for adolescents and 0-0.004 mg/kg bw/day for adults (consumers only). At the 95th percentile, exposure to BHT from chewing gum consumption is in the range of 0.004-0.008 mg/kg bw/day for children, 0.003-0.006 mg/kg bw/day for adolescents and 0.004-0.008 mg/kg bw/day for adults.

Using a worst case scenario of combined exposure to BHT from food categories where its use as food additive is authorised, the Panel estimated potential exposure to be in the range of 0.01-0.03 mg/kg bw/day on average and 0.03-0.17 mg/kg bw/day at the 95th percentile for adults. For adolescents, combined exposure to BHT from all food categories would be on average in the range of 0.01-0.05 mg/kg bw/day and in the range of 0.04-0.13 mg/kg bw/day at the 95th percentile. For children, combined exposure to BHT from all food categories would be on average in the range of 0.01-0.09 mg/kg bw/day and in the range of 0.05-0.30 mg/kg bw/day at the 95th percentile. Exposure to BHT from its use in food contact materials with a specific migration limit of 3 mg/kg food and the assumption that every day throughout lifetime a person weighing 60 kg consumes 1 kg of food packed in plastics containing BHT in the maximum permitted quantity would be increased by 0.05 mg/kg bw/day. Assuming that children weighing 15 kg also consume 1 kg of food packed in plastics containing BHT in the maximum permitted quantity, exposure to BHT of children would be increased by 0.2 mg/kg bw/day.

## 3. Biological and toxicological data

### 3.1. Absorption, distribution, metabolism and excretion

Absorption, distribution, metabolism and excretion of BHT have been studied in mice, rats, rabbits, chickens, monkeys and humans.

A single oral dose of 20 or 500 mg/kg bw $^{14}$C-BHT, labelled in the p-methylgroup, given to male and female DDY/Slc mice resulted in rapid absorption and distribution of $^{14}$C to the tissues, mainly stomach, intestines, liver and kidney. Excretion of $^{14}$C was mainly in the faeces (41-65% of the dose) and urine (26-50% of the dose), with lesser amounts in expired air (6-9% of the dose) (Matsuo et al., 1984). The half-life for these single doses in stomach, intestines, liver, and kidney was 9-11 hours. When daily doses were given for 10 days, the half-life for $^{14}$C in blood, liver, kidney, lung and testis was 5-15 days (Matsuo et al., 1984). The major metabolite in the urine was the glucuronide conjugate of the acid and the major metabolite in faeces was the free acid (Matsuo et al., 1984).

In a study in rats it was reported that accumulation of BHT is found in adipose tissue. After dietary exposure of rats for 35 days, the fat tissue concentration reached a maximum after 10 days (JECFA, 1996). The accumulation of BHT in human adipose tissue seems to be higher than in rats when compared on a dose/body weight basis (Anonymous, 2002; Madhavi et al., 1996); in humans, in contrast to rats, no considerable enterohepatic circulation was shown (JECFA, 1996).

More than 40 metabolites of BHT have been identified (Matsuo et al., 1984; JECFA, 1996). Of these, the most important are shown in Figure 2. In vivo biotransformation of BHT has been studied in rats, rabbits, and humans. In vitro studies, using mouse liver microsomes, showed formation of BHT quinone methides. In rats, metabolism was characterized by oxidation at one or both the *tert*-butyl groups, followed by glucuronide conjugation and excretion via urine or faeces. In rats, four major metabolites have been identified: 3,5-di-tert-butyl-4-hydroxy-benzoic acid (BHT-acid), 2,6-di-tert-butyl-4-hydroxymethylphenol (BHT-alcohol), and 3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-aldehyde), and BHT quinone methide (2,6-di-tert-butyl-4-methylene-2,5-cyclohexadien-1-one, BHT-QM) (Madhavi et al., 1996).

Madhavi et al. (1996) reported that in general, the oxidative metabolism of BHT was mediated by the microsomal monooxygenase system. In rats, rabbits, dogs and monkeys, oxidation of the p-methyl group predominated, whereas in humans the tert-butyl groups were oxidized. Oxidation of both the p-methyl and tert-butyl groups was observed in mice. 3,5-Di-tert-butyl-4-hydroxybenzoic acid (BHT-COOH) is a major metabolite formed by oxidation of the methyl group and may be generated via the corresponding alcohol and aldehyde (BHT-aldehyde).

The quinone methides formed in mice have been reported to be probably responsible for the lung damage seen in this species (Yamamoto et al., 1997).

Specifically, after parenteral dosing of rats (i.p and i.v.), four principal primary biliary metabolites were identified: 3-5-di-tert-butyl-4-hydroxy-benzoic acid, 3,5-di-tert-butyl-4-hydroxybenzaldehyde, 3-5-di-tert-butyl-4-hydroxybenzyl alcohol, and 1,2-bis(3,5-di-tert-butyl-4-hydroxyphenyl)ethane (BHT-dimer). The major metabolites in rat urine were 3,5-di-tert-butyl-4-hydroxybenzoic acid and S-(3,5-di-tert-butyl-4-hydroxybenzyl)-N-acetylcysteine. Free 3,5-di-tert-butyl-4-hydroxybenzoic acid was the major metabolite in rat faeces (JECFA, 1996).

Results from i.v. administration of BHT-acid or 2,6-di-*tert*-butyl phenol (DBP) to rats suggested that BHT-acid, considered as a main metabolic end-product of BHT, was metabolized to the quinone and hydroquinone following its decarboxylation to form DBP (JECFA, 1996).

After oral administration of a single dose of BHT to rabbits, the metabolites 2,6-di-tert-butyl-4-hydroxymethylphenol (BHT-alcohol), 3,5-di-tert-butyl-4-hydroxy-benzoic acid (BHT-acid), 4,4'-ethylene-bis-(2,6-di-tert-butylphenol) (BHT-dimer), and 3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-aldehyde) were identified in urine, whereas unchanged BHT was present only in the faeces (JECFA, 1996).

Three in vitro mechanistic biotransformation studies have been performed using microsomal preparations. The primary metabolites were the 4-hydroxymethyl product (BHT-$CH_2OH$) and a primary alcohol resulting from hydroxylation of one tert-butyl group (BHT-tert-BuOH), BHT-quinol

(2,6-di-tert-butyl-4-hydroxy-4-methyl-2,5-cyclo-hexadien-1-one), BHT-quinone (2,6-di-tert-butyl-p-benzoquinone), and BHT-quinone methide (2,6-di-*t*-butyl-4-methylene-2,5-cyclohexadien-1-one) (JECFA, 1996).

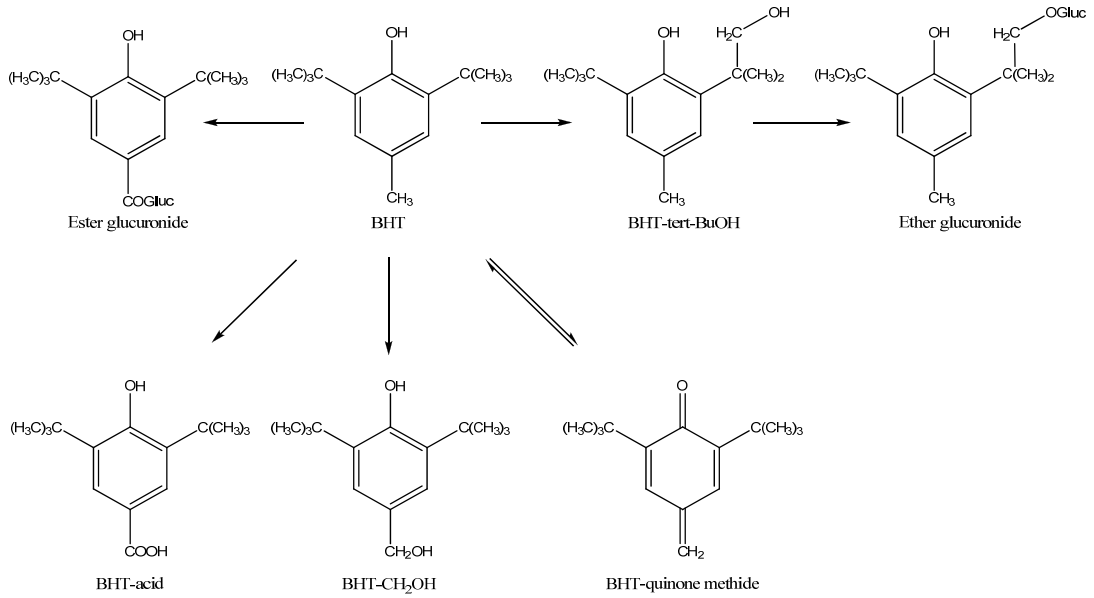


**Figure 2.** Major metabolites of BHT. Redrawn from Madhavi et al. (1996)

In Wistar rats (5 male and 5 female per group) given 0.5 and 1% BHT ad libitum in the diet (estimated to be equal to approximately 250 and 500 mg/kg bw/day) for up to 50 days, the concentration of BHT increased rapidly in liver and body fat. On average, a level of 30 mg BHT/kg body fat was observed in males and 45 mg BHT/kg body fat in females, and a level of 1-3 mg BHT/kg was observed in the liver. Upon return to normal diet the concentration in the tissues decreased with a half-life of 7-10 days. In two rats given single oral doses of $^{14}$C-labeled BHT (1100 mg/rat), nearly 80-90% was recovered in 4 days, with up to 40% in the urine. About 3.8% was retained in the tissues, 41.3% of which (i.e. 1.6% of original dose) was found in the small intestine. The excretion in bile was found to be 17-53% of the dose administered (Daniel and Gage, 1965).

The major urinary metabolites observed in rats were BHT-acid (3,5-di-tert-butyl-4-hydroxybenzoic acid) (both free and as a glucuronide) and BHT-mercapturic acid (di-tert-butylhydroxybenzyl acetyl cysteine) in addition to many other compounds including BHT alcohol (3,5-di-tert-butyl-4-hydroxybenzyl alcohol), BHT aldehyde (3,5-di-tert-butyl-4-hydroxybenzaldehyde), and BHT dimer. Free BHT acid was the major metabolite in faeces. About 10% of the dose was excreted unchanged. Tye et al. (1965) reported distinct sex differences in the mode of excretion. Female rats excreted 40-60% of a single oral dose in faeces and 20-40% in the urine. Males excreted 70-95% in the faeces and 5-9% in the urine. Females showed more tissue retention, especially in the gonads (Madhavi et al., 1996).

The major metabolites observed in rabbits were BHT alcohol, BHT acid, and BHT dimer. Excretion of all metabolites was essentially complete in 3-4 days. Urinary metabolites constituted 37.5% glucuronides, 16.7% sulphates, and 6.8% free phenols. Unchanged BHT was observed only in the faeces. Significant biliary excretion of BHT and metabolites has also been reported. In dogs, the metabolism was similar to that of rats, and significant biliary excretion was observed. In monkeys, the major metabolite was the glucuronide of BHT acid, and the rate of excretion was similar to that of humans (Madhavi et al., 1996). BHT and its metabolites were excreted primarily in the urine in humans, whereas in rats the metabolites of BHT were seen in urine and bile, irrespective of the route of administration (Anonymous, 2002).

In one study in humans the urinary metabolites BHT-COOH (the "acid") and benzoyl-glycine (hippuric acid) were found after two separate oral doses of 100 mg BHT, and in another study BHT-COOH and its glucuronide ester were the only major metabolites identified in urine after an oral dose of 1.0 g (JECFA, 1996).

In two men, excretion of a single oral dose of 40 mg/kg [$^{14}$C]BHT was 50% in the first 24 hours, followed by slower excretion for the next 10 days. In total, 63-67% of the dose was excreted in the urine after 10 days. Faecal excretion was measured in one man and was found to be 0.3% of the dose per day initially, and waning off to 0.02% of the dose per day, 31 days after dosing (Daniel et al., 1967).

In summary, these studies show that BHT is rapidly absorbed from the gastrointestinal tract and distributed. Upon absorption, BHT is generally distributed to the liver and body fat. Excretion is mainly via urine and faeces. The metabolism of BHT is complex. There may be important species differences. It is not known for example whether humans are capable of forming the quinone methides, metabolites that were found in rats and mice. In addition, biliary excretion seems not to be as significant in man as it is in rats, rabbits and dogs.

## 3.2. Toxicological data

### 3.2.1. Acute oral toxicity

According to the results of acute toxicity studies with BHT reported to JECFA (1996), BHT possesses low acute toxicity.

The acute oral $LD_{50}$ of BHT was 1700-1970 mg BHT/kg bw in rats, 2100-3200 mg BHT/kg bw in rabbits, 10 700 mg BHT/kg bw in guinea pigs, 940-2100 mg BHT/kg bw in cats, and 2000 mg BHT/kg bw in mice (Madhavi et al., 1996).

### 3.2.2. Short-term and subchronic toxicity

Short-term and subchronic toxicity studies in mice, rats and dogs have been reported to JECFA (1996) and various inconsistent findings were cited. Specifically, in a study in C3H mice (17-39 mice/group), a reported incidence of hepatocellular tumours induced by 10-month administration of diet containing 0, 0.05 or 0.5% BHT (estimated to be equivalent to approximately 0, 80 or 800 mg/kg bw/day) was not dose-dependent. Furthermore, when compared to data obtained from a study investigating the spontaneous occurrence of hepatic tumours in C3H mice it was concluded that there was no significant effect of BHT. The adrenal weight in rats was shown to be increased after feeding 0.5% in diet for 6 weeks and 0.1% in diet up to 16 weeks, without evidence of histopathological change. No conclusive NOAEL values have been derived for these effects. The Panel noted that the toxicological significance of these findings is uncertain and needs to be considered in the framework of the findings in long-term studies.

Takahashi (1992) reported a study in which male ddY mice received 0, 1.35%, 1.75%, 2.28%, 2.96%, 3.85% or 5.00% BHT in a purified diet, (reported to be equivalent to 0, 1570, 1980, 2630, 3370, 4980 or 5470 mg/kg bw/day, respectively) for 30 days. Absolute and relative kidney weights exhibited dose-related decreases and increases, respectively, which were related to a reduced body weight gain. Histopathology of the kidney revealed a dose-related increase in the incidence and severity of toxic nephrosis (0, 2, 3, 6, 8, 10, and 10 out of 10 mice/group) as indicated by a number of tubular lesions (distal and proximal tubular degeneration, distal tubular necrosis, distal tubular regeneration, tubular dilatation and cysts). JECFA, describing this study (JECFA, 1996), concluded that the Effective Dose in 50% of the population ($ED_{50}$) for toxic nephrosis in the male ddY mouse following 30 days of administration of BHT in the diet was calculated to be 2300 mg/kg bw/day.

The following additional studies have become available since the previous evaluations:

In a 28-day dietary exposure study the effects of BHT on plasma and tissue concentrations of α-tocopherol (α-T), γ-tocopherol (γ-T) and cholesterol were studied in a group of 8 male Sprague-Dawley rats (start body weight 60 g) and a control group also consisting of 8 male Sprague-Dawley rats. The rats were fed a restricted amount of feed containing 2 g BHT/kg in standardized diets and low but adequate levels of vitamin E. This corresponded to approximately 27.1 mg BHT/day or 350 mg/kg bw/day at the beginning of the experiment and 165 mg/kg bw/day at the end. BHT did not affect feed intake, but decreased the body weight ($p< 0.0005$), the amount of liver lipids and liver cholesterol ($p< 0.0001$), and increased the weight of livers ($p< 0.0001$) and lungs ($p< 0.05$) relative to body weight. α-T was elevated ($p< 0.0001$) and γ-T was reduced ($p< 0.005$) in blood plasma and liver. To investigate whether the α-T-sparing action of BHT was due to the inhibition of tocopherol-omega-hydroxylase, HepG2 cells were incubated with BHT in the presence of delta-tocopherol (delta-T) and the 3'- and 5'-delta-carboxychromanol metabolites in the media were analyzed by GC/MS. BHT did not inhibit tocopherol-omega-hydroxylase activity in hepatocyte cultures. The authors concluded that BHT markedly increased α-T concentrations in plasma and organs of Sprague-Dawley rats by a mechanism that apparently does not involve inhibition of tocopherol-omega-hydroxylase, a key enzyme in tocopherol catabolism (Frank et al., 2003).

BHT was fed ad libitum to groups of 10 male Sprague-Dawley rats (6-10 weeks old) at three concentrations: 0.2, 0.4 and 0.8% in the diet (estimated to be equivalent to dose levels of approximately 200, 400 and 800 mg/kg bw/day) for cumulative periods of 6, 12, 18 and 24 weeks, and the results were compared with results from corresponding groups treated with a potent carcinogen (0.5% 7,12-dimethylbenz(a)anthracene – DMBA) in the diet for 12 weeks, and with results from an untreated control group (Safer and Al-Nughamish, 1999). On week 6, BHT treatment resulted in a statistically significant ($p< 0.05$) increase in liver weight in all dose groups. In week 18 only the highest dose group had a statistically significant increased liver weight compared to the control, and in week 24 no such differences were found. When subjected to electron microscopy liver cells exhibited various degenerative features, such as “moth eaten” appearance, anucleation, degenerated nuclei, out-of-shape mitochondria and cytoplasmic disintegration. The statistically most significant ($p< 0.01$) differences in occurrence of hepatocellular hypertrophy were observed in week 18 for the two lowest dose groups. In comparison, the DMBA group’s hepatocyte cytoplasm was severely disintegrated. The Panel noted that there is no clear dose-response, and that a NOAEL for BHT cannot be derived from this study.

Al Akid et al. (2001) conducted a study to evaluate the nephrotoxic and pneumotoxic effects of BHT. Adult albino rats (n=75) were used and divided into a negative control group, vehicle control groups (received olive oil orally or i.p.), a group receiving orally 15 mg BHT/kg bw/day and a group receiving BHT i.p. at a dose of 400 mg/kg bw/week. The animals were sacrificed after 12 weeks. Blood samples were collected to analyze the following: blood urea nitrogen (BUN), serum creatinine, uric acid, glutamyl transferase and serum electrolytes (potassium and sodium). Tissue malondialdehyde, superoxide dismutase, glutathione peroxidase and selenium were also analyzed. The kidneys and lungs were examined histopathologically by both light and electron microscopy. BHT exposure in this study resulted in both nephrotoxicity and pneumotoxicity. No further details of this study can be obtained since only the abstract is available.

The microsomal enzyme profile was studied in 105 broiler chickens fed dietary BHT ad libitum at 130-2080 mg/kg feed) for 6 weeks. The increase ($p< 0.01$) in both hepatic microsomal enzymes cytochrome b5 and NADPH-dependent cytochrome P450 reductase was dose-dependent; in the highest BHT group, the maxima reached 250% and 162.5%, respectively (Rao et al., 1999). The authors subsequently reported that BHT feeding in chickens (same design as before) caused a marked congestion of the liver and kidney and diffuse enlargement of the liver with rounded borders and rupture with hemorrhage (Rao et al., 2000).

In summary, short-term or subchronic exposure to BHT affects the liver of mice, rats and chickens, also showing histopathological changes in this organ. In addition, BHT has been shown to increase the relative thyroid and adrenal weight in rats. Newer data show an α-tocopherol sparing effect in rats. None of the studies available could be used to derive a NOAEL.

### 3.2.3. Genotoxicity

The genotoxicity of BHT has been studied in several in vitro systems (JECFA, 1996) and an extensive database of genotoxicity studies for BHT was reviewed by Bomhard et al. (1992) and by Williams et al. (1999). These authors concluded that the majority of evidence indicated a lack of potential for BHT to induce point mutations, chromosomal aberrations, or to interact with or damage DNA, and that BHT does not represent a genotoxic risk.

TemaNord (2002) reported that BHT was not genotoxic in vivo in rats at oral doses up to 500 mg/kg bw (dominant lethal assay) or in mice at doses of 1% in the feed (approximately 1000 mg/kg bw/day) (dominant lethal and heritable translocation assays).

The following additional studies have been identified in the literature:

The mechanism of DNA damage by the BHT metabolites BHT quinone and BHT-OOH was investigated in a study in vitro in cellular and acellular systems. Based on the results obtained, the authors suggested that following metabolic conversion BHT may induce oxidative damage to DNA through two different pathways, i.e. the oxidation by BHT-OOH in presence of transition metals, and the intracellular generation of $H_2O_2$ by BHT-quinone (Oikawa et al., 1998).

Atta et al. (2007) reported no significant changes in the number of chromosomal aberrations in bone marrow cells after exposure of a group of 6 albino Swiss rats to 3 mg BHT/kg bw/day by gavage for 3 months.

Kim and Ryu (2007) evaluated the genetic toxicity of BHT using an in vitro mouse lymphoma *tk*+/- gene assay (MLA) and an in vivo mouse micronucleus (MN) assay. The genotoxicity of BHT was assessed at concentrations up to 40 or 50 μg/ml in the absence and presence of S-9 activation, showing no induction of gene mutations nor of chromosomal damage in the L5178Y thymidine kinase (tk)+/- mouse lymphoma assay. In addition, BHT was tested in an in vivo micronucleus (MN) assay with mouse peripheral reticulocytes. The frequency of MNRETs observed 48 hours after i.p. injection of single doses of 17.3, 34.5 and 69.0 mg/kg of BHT was not dose-dependently increased. It was concluded by the study authors that BHT did not show any clastogenic potential in the in vivo mouse micronucleus assay.

In general, the genotoxicity studies on BHT show that the majority of evidence indicates a lack of potential for BHT to induce point mutations, chromosomal aberrations, or to interact with or damage DNA. The Panel recognised that positive genotoxicity results obtained in vitro with BHT and BHT metabolites may be due to pro-oxidative chemistry giving rise to formation of quinones and reactive oxygen species and that such a mechanism of genotoxicity is generally considered to have a threshold.

### 3.2.4. Chronic toxicity and carcinogenicity

JECFA (1996) evaluated several chronic toxicity and carcinogenicity studies with BHT in mice and rats. These, and those found additionally in a literature search, are summarized below:

*Mice*

Six out of 18 male BALB/C mice fed dietary BHT ad libitum at a level of 0.75% (corresponding to a dose of approximately 750 mg/kg bw/day) for a period of 12 months developed marked hyperplasia of

the hepatic bile duct with an associated subacute cholangitis. This lesion was not seen in any of the 64 mice in the control group, in a group of 19 mice fed BHT + diethylnitrosamine in the drinking water at a total dose of 490 mg BHT/kg bw over a 7-week period, nor in historical controls (Clapp et al., 1973).

In an unpublished study submitted to JECFA (Brooks et al., 1976) mice (CFI strain, 48 mice per group) were maintained on diets containing 1000 mg BHT/kg feed. At week 4, one group was then fed a diet containing 2500 mg BHT/kg feed, and at week 8, another group was fed a diet containing 5000 mg BHT/kg feed. These dose levels of 1000, 2500 and 5000 mg/kg feed correspond to approximately 0, 100, 250 and 500 mg/kg bw/day, respectively. The mice were maintained on these diets until 100 weeks of age. There was an increased incidence of lung neoplasia in treated mice (Table 3). There were no morphological features to distinguish the lung tumours in treated mice from those in controls (Brooks et al., 1976) cf. (JECFA, 1996).

The Panel performed a BMD analysis on the data from Brooks et al. (1976) as reported by JECFA (1996) on the incidence of lung neoplasia in mice induced by BHT (Table 4). It was noted that when a larger number of animals were used by the same investigators, the findings were not confirmed (Clapp et al., 1978).

**Table 3:** Incidence of lung neoplasia in mice induced by BHT (Brooks et al. 1976 as reported by JECFA (1996)).

| Dose in mg/kg bw/day | No of animals | Tumour incidence (%) |
|---|---|---|
| 0 | 48 | 47 |
| 100 | 48 | 53 |
| 250 | 48 | 74 |
| 500 | 48 | 75 |

**Table 4:** Results of a BMD analysis of the data from Brooks et al. (1976) on the incidence of lung neoplasia in mice induced by BHT using BMDS version 2.1.2. software and the default settings of extra risk, a Benchmark Response (BMR) of 10%, a 95% confidence limit, but no restrictions.

| Model | No of parameters | Log likelihood | p value | Accepted | $BMD_{10}$ mg/kg bw/day | $BMDL_{10}$ mg/kg bw/day |
|---|---|---|---|---|---|---|
| null | 1 | -127.263 | | | | |
| full | 4 | -120.868 | | | | |
| logistic | 2 | -122.001 | 0.3220 | yes | 75.7747 | 53.9318 |
| probit | 2 | -122.051 | 0.3065 | yes | 78.3845 | 56.6575 |
| multistage | 2 | -122.158 | 0.2752 | yes | 58.0121 | 37.8085 |

Based on the BMD analysis using the data reported by Brooks et al. (1976) on the incidence of lung neoplasia in mice induced by BHT, the Panel derived a $BMDL_{10}$ of 38 mg/kg bw/day.

BHT was administered in the diet at concentrations of 0, 1 or 2% (dose corresponding to an average of 0, 1695 or 3805 mg/kg bw/day) to $B6C3F_1$ mice (10/sex/dose group; 20/sex in the control) for 104 weeks (Inai et al., 1988). All mice survived this period, except one male in the 1% group, and 3 females in the control group. After the treatment period, all the surviving mice were given basal diet for an additional 16 weeks (recovery period) prior to pathological examination. In male mice administered BHT, there was a statistically significant increase in the incidence of hepatocellular adenomas (19%, 38% and 53% in control, low- and high-dose groups, respectively), but hepatocellular

carcinomas were not increased (22%, 26% and 17% in control, low- and high-dose groups, respectively). Incidences of tumours in female mice were not significantly increased.

Groups of male and female C3H mice (17-39 mice/group), which were 6-10 weeks old at the beginning of the experiment, were maintained for 10 months on a semi-synthetic diet containing 0.05 or 0.5% BHT. Control groups were maintained on BHT-free semi-synthetic diet or commercial lab chow. At the end of the test period, the liver and lungs were excised and inspected grossly for proliferative lesions. Of the proliferative lesions considered to be clearly identifiable as tumours, approximately 50% were examined microscopically. Mice maintained on diets containing BHT had lower body weights than controls. Male mice fed BHT showed an increase in liver tumours, compared to controls. Histologically, the tumours were identified as hepatocellular adenomas. No increase was observed in female mice. The reported incidence of liver tumours in male C3H mice was 10/26 (38%), 15/26 (58%), 2/37 (5%) and 7/38 (18%) in the 0.5% BHT, 0.05% BHT, control BHT-free, and control lab chow groups, respectively. The Panel noted the limited number of tumours that were examined microscopically.

In a study in which C3H mice were maintained on diets containing 0.5% BHT for one month followed by lab chow for 10 months, or control diet (BHT-free) for one month followed by lab chow for 10 months, the incidences of liver tumours in the two groups of male mice were 3/35 (9%) and 5/29 (17%), respectively. Dietary BHT did not result in an increased incidence of lung tumours in either male or female C3H mice.

In another study in which male BALB/c mice were maintained for one year on a BHT-free diet, or diets containing 0.05% BHT or 0.5% BHT, the incidences of liver tumours were 4/30 (13%), 6/43 (14%), and 2/28 (7%) for the respective groups (Lindenschmidt et al., 1986).

*Rats*

A series of long-term studies have been performed in rats. No consistent adverse effects were shown (Deichmann et al., 1955; Hiraga, 1978; Hirose et al., 1981; NCI, 1979; Shibata et al., 1979; Williams et al., 1990a) cf. (JECFA, 1996).

Two long-term studies were published (Olsen et al., 1986; Price, 1994) indicating hepatotoxicity and effects on the thyroid. These studies were described by JECFA (1996) as follows:

In the study by Olsen (Olsen et al., 1986), groups of 60, 40, 40, or 60 Wistar rats of each sex ($F_0$ generation) were fed a semi-synthetic diet containing BHT at concentrations that resulted in intakes of 0, 25, 108, or 276 mg/kg bw/day for male rats and of 0, 26, 106 and 287 mg/kg bw/day for female rats, respectively. The $F_0$ rats were mated after 13 weeks of treatment. The $F_1$ groups consisted of 100, 80, 80, and 100 $F_1$ rats, respectively, of each sex from the offspring from each group. Because of an adverse effect on the kidney in the parents, the concentration of BHT in the highest dose group was lowered to 250 mg/kg bw/day in the $F_1$ generation. The study was terminated when rats in the $F_1$ generation were 144 weeks of age.

The Panel noted that 144 weeks is longer than the duration indicated by current guidelines for these studies and that this may influence the outcome.

The number of litters of ten or more pups at birth decreased with increasing BHT dose with the number of pups/litter amounting to 10.9, 9.6, 10.3 and 9.1 at increasing dose levels. The Armitage-Cochran test for linear trend in proportions demonstrated that the fraction of litters with ten or more pups decreased significantly with BHT dose ($p<0.001$). At weaning, treated $F_1$ rats showed a dose-dependent reduction in body weights compared to the controls. In the low, mid, and high-dose groups, the reductions in body weight were for males/females 7%/5%, 11%/10%, and 21%/16%. Food intake was comparable for all groups. The survival of BHT-treated male and female $F_1$ rats was significantly and dose-dependently better than that of the controls. In both sexes differences ($p < 0.001$) in

longevity were seen. It was reported that no BHT-related changes were observed in haematology parameters. $F_1$ females of the highest dose group showed an increase in serum cholesterol and phospholipids, and serum triglycerides were reduced in the high-dose group for both sexes.

Histopathological examinations indicated dose-related increases in the numbers of hepatocellular carcinomas in male rats and an increase in hepatocellular adenomas in both male and female rats. At increasing dose levels the incidences of adenomas were 1/100, 1/80, 5/80 and 18/99 ($p< 0.001$ at the highest dose group and test for trend $p< 0.001$) for males and 2/100, 3/79, 6/80 and 12/99 (non-significant but test for trend $p< 0.05$) for females. The incidences for carcinomas were 1/100, 0/80, 1/80 and 8/99 ($p< 0.05$ at the highest dose group and test for trend $p< 0.01$) for males and 0/100, 0/79, 0/80 and 2/99 (non-significant) for females. $F_1$ rats in which hepatocellular tumours were detected were all more than 2 years old. Tumours were also found in other organs of some of the treated rats, including thyroid, pancreas, ovary, uterus, thymus, reticulo-endothelial system, and mammary gland, but their incidence was not statistically significantly different from that in controls. The JECFA report analysing this study (JECFA, 1996) indicated that the spontaneous incidence of hepatocellular neoplasms was usually less than 3% in Wistar rats from the laboratory performing this study, as well as other European laboratories (Solleveld et al., 1984; Deerberg et al., 1980; Olsen et al., 1986).

The Panel concluded, based on the study of Olsen et al. (1986), that the NOAEL for non-neoplastic effects was 25 mg/kg bw/day, based on the effects on litter size, sex ratio and pup body weight gain during the lactation period, in the reproduction segment of the study.

Table 5 presents the data for the study of Olsen et al. (1986) on the hepatocellular carcinomas in male and female rats exposed to BHT, and Table 6 presents the results from a BMD analysis performed by the Panel of the data from Olsen et al. (1986) on the incidence of hepatocellular carcinomas in male rats induced by BHT.

**Table 5:** Incidence of hepatocellular carcinomas in male and female mice induced by BHT (Olsen et al. 1986).

| Gender | Dose in mg/kg bw/day | No of animals | Tumour incidence (no of animals) |
|---|---|---|---|
| male | 0 | 100 | 1 |
| | 25 | 80 | 0 |
| | 108 | 80 | 1 |
| | 276 | 99 | 8 |
| female | 0 | 100 | 0 |
| | 26 | 79 | 0 |
| | 106 | 80 | 0 |
| | 287 | 99 | 2 |

**Table 6:** Results of a BMD analysis of the data from Olsen et al. (1986) on the incidence of hepatocellular carcinomas in male rats induced by BHT using BMDS version 2.1.2. software and the default settings of extra risk, a Benchmark Response (BMR) of 10%, and a 95% confidence limit.

| Model | No of parameters | Log likelihood | p value | accepted | $BMD_{10}$ mg/kg bw/day | $BMDL_{10}$ mg/kg bw/day |
|---|---|---|---|---|---|---|
| null | 1 | -45.6668 | | | | |
| full | 4 | -38.7690 | | | | |
| logistic | 2 | -39.4984 | 0.4822 | yes | 303.806 | 255.086 |
| probit | 2 | -39.5495 | 0.4582 | yes | 311.004 | 251.523 |
| multistage | 2 | -39.4634 | 0.4994 | yes | 324.976 | 247.249 |
| Weibull | 3 | -39.3702 | 0.2728 | yes | 307.512 | 248.451 |

Based on the BMD analysis using the data reported by Olsen et al. (1986) on the incidence of hepatocellular carcinomas in male rats induced by BHT, the Panel derived a $BMDL_{10}$ of 247 mg/kg bw/day.

The Robens Institute (Price, 1994) described a long-term study initiated in order to investigate the role of hepatic changes in the development of hepatocellular carcinomas in rats following in utero/lifetime exposure to BHT. The dosing regimen of the study and the strain of rat used were similar to those in the 2-generation study described by Olsen et al. (1986). In this study reported by Price (1994), groups of 6 male and 48 female Wistar rats, aged 13 weeks and 9 weeks, respectively, were fed BHT in the diet at doses of 0, 25, 100 or 500 mg/kg bw/day for 3 weeks prior to mating (in the study of Olsen et al., 1986 this period was 13 weeks). The rats were then mated. On day 20 of gestation, 5 pregnant rats were sacrificed for assessment of body and liver weights and liver histopathology. The pups were delivered by caesarean section and retained for assessment of a number of parameters. The remaining females (20 dams in the control and high-dose groups; 24 dams in the low- and mid-dose groups) were allowed to deliver normally. On day 6 post- partum, litters were either culled or augmented to comprise 8 pups, at the same time maximizing the number of robust males in each litter. At weaning (3 weeks), 4 pups from each of 5 litters per group were selected randomly, maximizing the numbers of males, for assessment of a number of parameters. The male pups from the remaining litters (approximately 60/group) were selected to continue in the study and were placed in one of 4 groups corresponding to the diets fed to their parents, with the exception that the high dose was reduced to 250 mg/kg bw/day a level also used for the high dose exposure in the Olsen study (Olsen et al., 1986). Interim kills were conducted at 1, 6, 11, or 16 months. The study was terminated 22 months after the $F_1$ male rats were placed on test diets.

In the first 5 weeks of BHT administration, a reduction in body weight gain was noted in the high-dose males. Body weight gain in all other treatment groups was similar to that in controls. During pregnancy and lactation, there was no difference in food consumption between treated and control female rats, and body weights of the dams were similar at weaning. At the sacrifice on day 20 of gestation, both absolute and relative liver weights of the dams were increased in a dose-related manner, statistically significant at the high dose. The body weights of the females, both including and excluding their litters, were similar in all groups. Histopathological examination of the liver revealed mild enlargement of centrilobular hepatocytes and eosinophilia in 4/5 high-dose animals, and 1/5 low-dose animals, consistent with induction of mixed-function oxidase activity. A decrease was noted in the mitotic index of hepatocytes from dams receiving 100 and 500 mg/kg bw/day. No effect of treatment was evident on reproductive parameters including mating index, gestation index and viability index. The absolute numbers of resorptions/dam were also similar in treated and control groups. There was a slight decrease in the numbers of pups/litter in the low and high-dose groups, but

a dose-related trend was not observed. Body weights of the pups from the high-dose group were significantly lower than controls at birth (10%), and at days 6 (12%) and 21 (21%) of lactation. Mortality of the pups between culling and day 21 of lactation was 2%, 8%, 12% and 15%, in order of increasing dose. Body weights of the $F_1$ males were lower in the high-dose group, compared with controls, throughout the 22-month treatment period. During the first year of the study, the difference was in the range of 10-20%. Lower body weights, which differed from controls by about 5% were also noted in the mid-dose animals during the first half of the treatment period. No adverse reactions to treatment or effects on food consumption were noted. At the scheduled sacrifices, dose-related increases were observed in relative, but not absolute liver weights; the differences were statistically significant at the high dose.

A dose-related incidence of enlargement and eosinophilia of the centrilobular hepatocytes was observed at the scheduled sacrifices, starting at 6 months. This was indicative of proliferation of the smooth endoplasmic reticulum, consistent with an induction of mixed-function oxidases. Immuno-histochemical staining of liver sections from control and high-dose rats revealed a marked increase in hepatocellular content and distribution of cytochrome P450 2B with BHT treatment which persisted throughout the study. Histochemical staining revealed a marked induction of gamma-glutamyl trans-peptidase (GGT) activity in the periportal hepatocytes of nearly all of the high-dose rats, starting at 11 months of treatment. At 22 months, there was a higher incidence of eosinophilic and basophilic foci in the high-dose group. Histochemical staining of liver sections revealed a small number of high-dose animals with glucose-6-phosphatase- deficient AHF (altered hepatocellular foci) which was statistically significant. At 22 months, there was also a significant increase in the number of rats with hepatic nodules in the high-dose group (6/19 animals compared with none in the other groups).

No increases in the rate of hepatocellular proliferation were detected as a result of BHT administration at any point in the study commencing from 4 weeks post-weaning. It is of interest to note that Clayson et al. (1993) observed an increase in hepatocellular proliferation between 2 and 4 days after initiation of treatment of male Wistar rats with 0.5% dietary BHT. Such a transient increase would be difficult to detect with the widely spaced sampling times used in this study.

Total cytochrome P450 content was increased by 30-60% in the high-dose animals starting at 21 days of age. Dose-related increases were noted in epoxide hydrolase, glutathione-S-transferase and pentoxyresorufin-O-depentylase (PROD) activities, starting at 21 days of age, which were statistically significant in the mid- and high-dose groups. The increases in PROD activity were large, 10-25 fold in the mid-dose, and 20-80 fold in the high-dose groups.

Histopathological examination of the kidneys revealed a reduction in severity of chronic progressive nephropathy which affected the rats in all groups from 11 months. No effects on the adrenal were noted, although in a nearly identical, but invalidated study conducted in the same laboratory (Robens Institute, 1989) cytomegaly of cells of the zona fasciculata was observed in the mid- and high-dose groups at weaning and at 4 weeks post-weaning, but not at subsequent time points. In the present study, histopathology of the adrenal was conducted starting at 11 months post-weaning. Evidence of thyroid hyper-activity, characterized by reduction of follicular size, absence or reduction of colloid, irregularities in the follicular outline, hyperaemia and increase in the number of follicular cells was noted starting at 11 months in both the mid-dose group (mild changes affecting 75-82% of the rats) and the high-dose group (marked changes affecting 100% of the rats). Serum thyroxin levels in treated rats did not differ from controls.

The demonstrated effects on hepatic enzyme induction and consequent thyroid hyperactivity in the mid- and high-dose groups, together with the tumour data from the Olsen study, suggested a NOAEL of 25 mg/kg bw/day.

The International Agency for Research on Cancer (IARC) evaluated BHT (1987) and classified it in group 3, since no evaluation could be made of the carcinogenicity of BHT to humans, and there was limited evidence for the carcinogenicity in experimental animals. Williams et al. (1999) have reviewed

data on genotoxicity and carcinogenicity of BHT including reports which appeared subsequent to the IARC evaluation. Williams et al. (1999) argued that BHT is not genotoxic or carcinogenic; they particularly argued that the carcinogenicity in rats found in the study by Olsen et al. (1986) has not been confirmed in other studies with rats, and that the effects found may be attributable to study conditions and not to the administration of BHT. They also point out that a more recent study (Takagi et al., 1994) dosing Wistar rats for up to 18 months with 0.1% 2,2'-methylenebis (4-methyl-6-tert-butylphenol), an antioxidant which is essentially two molecules of BHT and has all the attributes of BHT, did not result in a carcinogenic response.

In addition to the above mentioned assessments and reviews, further studies on chronic toxicity and carcinogenicity have been identified.

Three groups of 8 male ICR mice were fed either 0, 50 or 500 mg BHT/kg bw/day in the diet for 12 months. No information was found whether feeding was ad libitum or not. The highest dose group, but not the lower, showed increased liver weight ($p<0.05$). The activity of intestinal glutathione-S-transferase (GST) in the highest dose group was almost twice as high as that of control mice ($p<0.05$). BHT markedly enhanced the hepatic GST activity compared with other groups ($p<0.05$). Increased lipid peroxidation by BHT could not be excluded (Jang et al., 1999).

***Special studies on potentiation of cancer***

A number of studies investigated the effect of BHT on the carcinogenic effects of other chemicals.

Several studies have been evaluated in 1995 by JECFA (1996). These are studies in bladder, gastrointestinal tract, liver, lung, mammary gland, pancreas, and skin as investigated in mice, rats, or hamsters. In a safety assessment by Williams et al. (1999) many of the references reported by JECFA (1996) and some additional studies are reviewed. Additional study reports are mentioned below.

Several reports indicated neoplasia-promoting activity of BHT when given after an initiating carcinogen for mouse lung (Witschi and Cote, 1977), colon (Lindenschmidt et al., 1986), rat liver (Maeura and Williams, 1984), and urinary bladder (Imaida et al., 1983). Consistent with these observations, BHT inhibits intercellular molecular transfer (Williams et al., 1990a; Williams et al., 1990b), a property of neoplasm-promoting agents (Budunova and Williams, 1994; Trosko et al., 1990; Williams, 1981; Yamasaki, 1996).

However, in one study, BHT (5000 mg/kg) had no promoting or syncarcinogenic effect on diethyl-nitrosoamine-induced mouse liver neoplasia by 38 weeks, whereas under the same conditions, phenobarbital (500 mg/kg) acted as an enhancer (Tokumo et al., 1991).

The tumour-promoting activity of BHT was demonstrated in transgenic mice carrying the c-Ha-ras gene (rasH2 mice) (Umemura et al., 1999, 2001, 2002 and 2006). When BALB/c mice were treated on day 17 of gestation with 3-methylcholanthrene (a known carcinogen in mice exposed in utero) and after birth with 6 weekly i.p. injections of 0 or 200 mg BHT/kg bw for 6 consecutive weeks, no statistically significant promoting effect of BHT exposure was observed on either tumour incidence, tumour multiplicity or the mutational spectrum produced in the Ki-ras gene (Gressani et al., 1999).

BHT was found to cause inflammation only in the promotion sensitive BALB/cByJ mouse, but not in the promotion resistant CXB4 mouse (Bauer et al., 2001a and 2001b). This could be due to differences in expression of toll-like receptors (Bauer et al., 2005).

The lung tumour-promoting action of BHT may be due to apoptosis of the non-neoplastic cells induced by the metabolite BHTOH. This was proposed by Dwyer-Nield et al. (1998) after experiments in mouse and human lung cell lines. Palomba et al. (1999) also found that exposure of U937 cells to BHT, unlike exposure to other antioxidants, promotes a time- and concentration-dependent induction of apoptosis. Apoptosis appeared to be causally linked to an altered cellular redox state in which

hydrogen peroxide plays a pivotal role. The Panel noted that this may reflect a pro-oxidant effect of BHT, which may occur at high concentrations of BHT.

Sun et al. (2003) applied a model system to investigate the promotion phase of pulmonary carcinogenesis. Previous studies (Thompson et al., 2001) strongly suggested that this activity is due to the cytochrome P450-catalyzed formation of quinone methides. The effects of these electrophiles on non-neoplastic C10 and E10 epithelial cell lines derived from a normal mouse lung explant were compared with effects on their corresponding neoplastic siblings, the A5 and E9 spontaneous transformants, respectively. The neoplastic cells were more resistant to cell killing, with $LC_{50}$ values of 165-180 μM for BHT-QM and 12-22 μM for BHTOH-QM, versus $LC_{50}$ values in the non-neoplastic cells of 105-118 μM and 5-6 μM, respectively. Constitutive reduced glutathione (GSH) concentrations were 12-20 nmol/$10^6$ cells, and BHT-QM toxicity was enhanced more than 2-fold by depleting GSH with buthionine sulfoximine (BSO). Formation of the GSH conjugate of BHT-QM accounted for a substantial fraction of the cellular GSH lost by quinone methide exposure. Enhanced lipid peroxidation and superoxide formation occurred in all cell lines treated with BHT-QM, but both neoplastic lines contained higher levels of GSH S-transferase and superoxide dismutase (SOD) activities. According to Sun et al. (2003) these data suggest that BHT-derived quinone methides may exert their promoting effects by inducing oxidative stress; such stress is better tolerated by neoplastic cells, which have higher levels of antioxidant enzymes. Normal cells are destroyed more readily, which allows neoplastic cells to expand their proliferation.

Gap junctional intercellular communication (GJIC) was inhibited by BHT in mouse lung epithelial (C10) and rat liver epithelial (WB-F344) cell lines at BHT concentrations of 150 μM and 62.5 μM, respectively, after 4 hours treatment. Inhibition occurred within 15-30 min and was reversed by removing BHT from the culture medium. The highly toxic BHT metabolite 6-t-butyl-2-(hydroxy-t-butyl)4-methylphenol (BHTOH) and the non-toxic BHT metabolite, 2,6-di-t-butyl-4-hydroxymethylphenol (BHTBzOH) were also tested. In both cell lines BHTOH was a more potent inhibitor of GJIC than BHT, whereas BHTBzOH was ineffective (Guan et al., 1995).

The Panel concluded that BHT in high doses can exert tumour-promoting effects in some animal models. The data do not allow the establishment of a NOAEL for a carcinogen-promotional effect.

In conclusion, lung or liver tumours have been seen in some studies in mice or rats exposed orally to BHT as the single test substance. BMD analyses of the data reported by Brooks et al. (1976) on the incidence of lung neoplasia in mice induced by BHT revealed a $BMDL_{10}$ of 38 mg/kg bw/day, and of the data reported by Olsen et al. (1986) on the incidence of hepatocellular carcinomas in male rats induced by BHT a $BMDL_{10}$ of 247 mg/kg bw/day. The NOAEL for non-neoplastic effects in the study of Olsen et al. (1986) was 25 mg/kg bw/day, based on the effects on litter size, sex ratio and pup body weight gain during the lactation period in the reproduction segment of the study.

### 3.2.5. Reproductive and developmental toxicity

Studies on reproductive toxicity have been reported in mice, rats, chickens and monkeys (JECFA, 1996). These are summarized below:

*Mice*

Diets containing 0, 0.1 or 0.5% (approximately 0, 150 or 750 mg/kg bw/day) BHT in feed containing 10% or 20% lard were given to an outbred random albino strain of mice 64 to 92 days before birth. The only statistical differences appeared at the 0.5% level of BHT when the figures for the two levels of lard were pooled. Here BHT increased the length of time to birth and at 12 days after birth the mean number of pups alive, the mean pup weight and the mean total litter weight were lower than the overall average (Johnson, 1965). Continuous ingestion of 0.5% BHT in the diet (750 mg/kg bw/day) by pregnant Swiss-Webster mice and their offspring also resulted in a variety of behavioural changes of offspring such as decreased sleeping, increased social and isolation-induced aggression and a severe

deficit in learning. No measures of reproductive parameters were made (Stokes and Scudder, 1974). These studies were also available to JECFA for their last assessment (JECFA, 1996). The Panel derived a NOAEL of 150 mg/kg bw/day from the first study.

A 3-generation study was carried out by Tanaka et al. (1993) in Crj:CD-1 mice. BHT was administered in the diets to 80 mice (10/sex/group) at dietary levels of 0.015, 0.045, 0.135, and 0.405%, resulting in doses of 0, 20, 70, 200 or 610 mg BHT/kg bw/day starting at 5 weeks of age. At 9 weeks of age the mice were mated. Body weight gain was consistently reduced from day 7 to 21 of lactation in the $F_1$ high-dose pups, but no body weight differences were noted in the $F_2$ pups compared with controls. In the $F_2$ males, lower scores were assigned to the treated groups for the 180° turn in the open field trial but this effect was not apparent in the $F_2$ female pups. No overt effects on reproduction have been reported in mice. The Panel noted the small group size in this study. The Panel established that the NOAEL from the Tanaka study would be 200 mg/kg bw/day.

*Rats*

A number of older studies exist in rats, none of which showed consistent dose-related effects on reproductive parameters, except the study by Olsen et al. (1986), already described in the section on chronic toxicity and carcinogenicity above. This study gave a NOAEL of 25 mg/kg bw/day, based on the effects on litter size, sex ratio, and pup body weight gain during the lactation period in the reproduction segment of a 2-generation the study.

In a special study on embryotoxicity of BHT, no significant embryotoxic effects were observed on examination of the skeletal and soft tissues of the fully developed fetuses as well as by other criteria (JECFA, 1996).

The unpublished 2-generation study in rats by Price (1994) is described extensively in the section on chronic toxicity and carcinogenicity above. No effect of treatment was evident regarding mating index, gestation index, viability index or resorptions/dam. Because culling of pups at day 6 was performed in a non-random manner, the reproductive function in BHT-treated animals could not be assessed. However, body weights of the pups of the high dose group (500 mg/kg bw/day) were statistically significantly lower than those of controls at birth (10%) and at day 6 of lactation (12%). The same study has later been published by McFarlane et al. (1997) who suggested that growth retardation observed in the pups could be due to inadequate milk production. Additionally, the Panel noted that the dams giving birth to the pups which showed growth retardation, showed liver enlargements and induction of pentoxyresorufin O-dependentylase and glutathione S-transferase. No NOAEL for reproductive and developmental toxicity could be established from this study.

Monkeys

A group of six adult female rhesus monkeys were maintained on a diet containing a mixture of BHT and BHA. The daily intake corresponded to 50 mg BHT/kg bw/day and 50 mg BHA/kg bw/day. Another group of six adult female rhesus monkeys were used as controls. The monkeys were fed the diet for one year prior to breeding and then for an additional year, including a 165-day gestation period. Haematology studies including haemoglobin, haematocrit, total and differential white blood cell (WBC) counts, cholesterol, Na+, K+, total protein, serum glutamic-pyruvic transaminase (GPT) and serum glutamic-oxaloacetic transaminase (GOT), were carried out at monthly intervals. Body weights were recorded at monthly intervals. Records of menstrual cycles were maintained through the test period. After a year of exposure, the females were mated with males that had received "control diet". During pregnancy complete blood counts were done on days 40, 80, 120 and 160 of gestation and on days 30 and 60 post-partum. The gestation of test animals was free of complications and normal offspring were delivered. A total of five offspring were born of the experimental monkeys and six of the control monkeys. Haematological evaluations were made on infants of the test and control monkeys at days 1, 5, 15, 30 and 60, and observations of the infants were continued through two years of age. Two experimental and two control infants, 3 months of age, were removed from their mothers

for a month of home cage observations. No clinical abnormalities were observed in parent or offspring during the period of study. Adult females continued to have normal offspring. Offspring born during the exposure period remained healthy, with the exception of one infant that died from unrelated causes. Home cage observations at the third month of life did not reveal any behavioural abnormalities (Allen, 1976). The Panel concluded that a NOAEL from this study would be 100 mg/kg bw/day of BHA and BHT combined, the only dose level tested.

*New studies*

Since the JECFA (1996) review only one new study on reproductive and developmental toxicity has been reported.

In a study by Eriksson and Siman (1996), pregnant diabetic and normal Sprague-Dawley rats were fed ad libitum either a standard diet or a diet with 1% of BHT (estimated to be equivalent to approximately 500 mg/kg bw/day). The fetuses of the diabetic rats (2.70 g body weight) were smaller than the fetuses of the normal rats (3.68 g) when the mothers were fed a standard diet. The BHT diet increased the fetal weight in the offspring of diabetic rats (3.17 g), with no change in fetuses of the normal rats (3.65 g). The placentas of diabetic rats were heavier than the placentas of normal rats; this difference was not present in the BHT-fed rats. The BHT treatment had no effect on the rate of resorptions, which was increased in the diabetic rats compared with the normal rats. In contrast, the increased rate of congenital malformations in the offspring of diabetic rats (19%), compared with that in the normal rats (0%), was markedly decreased by the BHT diet (2.3%). No malformations were found in the normal rats treated with BHT. The Panel considered that the only dose level of BHT tested in this study of 500 mg/kg bw/day can be considered a NOAEL.

### 3.2.6. Hypersensitivity, allergy and intolerance

Two patients with chronic idiopathic urticaria were subjected to double-blind, placebo-controlled, oral challenges with a series of food additives. During testing, BHT and BHA were identified as causative agents. Avoidance of foods containing BHT and BHA resulted in long-term reduction in severity and frequency of urticarial episodes (Goodman et al., 1990).

In a double-blind placebo controlled study by Hannuksela and Lahti (1986) with challenge tests of 44 patients with chronic urticaria, 91 with atopic dermatitis and 123 with contact dermatitis, none reacted to BHT when it was ingested in a capsule containing 50 mg BHT.

Signs of contact dermatitis after dermal exposure to BHT and allergic reactions after oral intake of a mixture of BHT and butylated hydroxyanisole (BHA) were occasionally reported (DFG, 1985; Flyvholm and Menne, 1990). In another report (Goodman et al., 1990), two patients with chronic idiopathic urticaria developed exacerbations when challenged with BHT/BHA, but had less symptoms after consuming a BHT/BHA-free diet.

When patch-tested on more than 15 individuals, BHT showed mild skin irritation; a positive skin reaction 14 days later was interpreted as sensitization (Mallette and von Haam, 1952).

Recent patch test results obtained from the medical surveillance of great numbers of workers (de Boer et al., 1989; Goh and Ho, 1993) or patients (Kanerva et al., 1997; 1999) were all negative.

The Panel noted that these limited reports do not allow any conclusions to be drawn about sensitization to BHT following oral intake.

### 3.2.7. Other studies

***Human studies: case studies***

Shilian and Goldstone (1986) reported a case of gastritis caused by ingestion of BHT in a 22-year-old woman who ingested 4 g of BHT on an empty stomach two days before the onset of the gastritis. This amounts to an acute dose of about 67 mg/kg bw assuming a body weight of 60 kg. Later that evening she experienced severe epigastric cramping, generalized weakness, nausea and vomiting, followed by dizziness, confusion and a brief loss of consciousness.

A similar case study was reported by Grogan (1986). A 24-year-old woman complained of light-headedness, unsteadiness of gait and slurred speech. On examination the following findings were noted: dysarthria, wide-based gait, a positive Romberg test, slowed mentation without thought disorder and dysmetria of the left (non-dominant arm). On the evening before admission the patient ingested 80 grams of BHT suspended in safflower oil on an empty stomach. This dose is estimated to be equivalent to about 1.3 g/kg bw, assuming a 60 kg body weight.

***Human studies: Epidemiological studies***

The association between dietary intake of BHT and stomach cancer risk was investigated in the Netherlands Cohort Study (NLCS) that started in 1986 among 120 852 men and women aged 55-69 years. A semi-quantitative food frequency questionnaire was used to assess food consumption. Information on BHT content of cooking fats, oils, mayonnaise and other creamy salad dressings and dried soups was obtained by chemical analysis, a Dutch database of food additives (ALBA) and the Dutch Compendium of Foods and Diet Products. After 6.3 years of follow-up, complete data on BHA and BHT intake of 192 incident stomach cancer cases and 2035 sub-cohort members were available for case-cohort analysis. Mean intake of BHT among sub-cohort members was 0.351 mg/day. No association with stomach cancer risk was observed for consumption of mayonnaise and other creamy salad dressings with BHT. A statistically non-significant decrease in stomach cancer risk was observed with increasing BHA and BHT intake. No significant association with stomach cancer risk was found in this study, for normal dietary intake of low levels of BHT (Botterweck et al., 2000).

***Mechanistic studies***

Groups of 6-7 male ddY mice treated orally with a single dose of BHT (200-800 mg/kg bw) in combination with an inhibitor of glutathione (GSH) synthesis, developed a hepatotoxic response which was both time- and dose-dependent. BHT alone (up to 800 mg/kg bw) produced no evidence of liver injury. The results suggested that BHT was activated by a cytochrome-P450-dependent metabolic reaction and that the hepatotoxic effect was caused by inadequate rates of detoxification of the reactive metabolite in mice depleted of hepatic GSH by administration of the inhibitor. Based on studies with structural BHT analogues, the authors suggested that a BHT-quinone methide may play a role in the hepatotoxicity in mice. The study authors concluded that normally BHT is converted, in a reaction mediated by cytochrome-P450 enzymes, to a reactive intermediate, which is detoxified by conjugation with GSH. Chemicals which depress GSH allow the toxicity of the reactive intermediate to become manifest (Mizutani et al., 1987).

A large number of studies are available (JECFA, 1996) but do not demonstrate consistent dose-related adverse effects following oral exposure. One paper (Malkinson et al., 1989) offers an explanation for BHT-induced pulmonary toxicity in all inbred strains of mice but not in rats. They found that the metabolite BHT-OH (BHT hydroxylated only on one tert-butyl group) was a major product of mouse liver and lung microsomes but not of rat microsomes. Further, Bolton et al. (1990) found that a quinone methide metabolite of BHT formed subsequent to hydroxylation of the tert-butyl side group was responsible for pulmonary toxicity in mice. Kupfer et al. (2002) further explored the effect of quinone methides by using an analogue to BHT-OH, 2-tert-butyl-6-(1'-hydroxy-1'-methyl)ethyl-4-methylphenol (BPPOH), that is structurally very similar to BHT-OH but forms a quinone methide (BPPOH-QM) capable of more efficient intramolecular hydrogen bonding and, therefore, higher electrophilicity than BHTOH-QM.

Chevillard et al. (2010) found that both wild type and Nrf3 (NF-E2-related factor 3) deficient mice were sensitive to a single administration of 400 mg BHT/kg bw by gavage. All the mice exhibited respiratory distress and considerable body weight loss following treatment. At the time of sacrifice, the BHT-treated Nrf3-/- mice had lost significantly more body weight than their wild type counterparts. In the lung, transcript levels of the transcription factors Nrf1, Nrf2 and Nrf3 were differentially regulated by BHT treatment. These data indicate that BHT may be toxic to the lungs of mice, probably because of the formation of quinone methides, which inhibit carbonyl reductase, which in turn leads to glutathione depletion and lack of detoxifying capacity. Lung tumour promotion by BHT is seen in some mouse strains, and this may be due to activation of proto-oncogenes.

***Special studies on haemorrhagic effects***

The SCF (1989) concluded that a series of studies has shown that some, but not all species tested show haemorrhage and/or a reduction in blood prothrombin index after dosing with BHT. The mechanisms by which BHT brings about these effects appear to be several but the major effect is a reduction in activity of certain clotting factors, principally those which are vitamin K-dependent. The most susceptible species for hemorrhagic effects appears to be the rat, and for this species the NOAEL for transient reduction (1 week duration) of the prothrombin index was approximately 9 mg/kg bw/day, and for persistent reduction (4 week duration), approximately 250 mg/kg bw/day given with the diet. Chickens may also respond with haemorrhage of the liver (Rao et al., 2000).

Administration to groups of five male Slc:ddY mice of 0.5%, 1.0% or 2.0% BHT in a purified diet (equivalent to 660, 1390 or 2860 mg/kg bw/day) for 21 days resulted in a dose-related increase in the mortality due to massive haemorrhage in the lungs (JECFA, 1996; Takahashi, 1992).

Male Wistar rats given BHT (3000 mg/kg bw/day) for up to 21 days, in a diet containing a minimum of 3 mg vitamin K3/kg (six times the recommended requirement), showed decreased vitamin K-dependent blood clotting factor activities, demonstrated by increases in factor-specific clotting time assays. Clotting times were prolonged within 7 days, statistically significant increased within 14 days ($p<0.001$) and maximally increased 5.5-fold at 21 days ($p<0.05$). Supplementation with a diet containing 250 mg vitamin $K_3$/kg reversed this effect. This study confirmed an antagonistic effect of BHT on vitamin K in rats, which resulted in a reduction in blood-clotting factors even when the diets contained adequate vitamin K. The authors of the study pointed out that this was a high-dose phenomenon with a threshold and a steep dose-response curve (Cottrell et al., 1994).

In summary, data indicate that exposure to high concentrations of BHT may cause haemorrhage as a consequence of antagonism to vitamin K in mice and rats.

***Special studies on effects on the thyroid***

Rats were fed 0, 500 or 5000 mg BHT/kg of feed (estimated to be equivalent to approximately 25 or 250 mg/kg bw/day) for 8, 26 or 90 days and the uptake of $^{125}I$ by the thyroid was determined. The presence of BHT in the diet resulted in a marked increase in the uptake of $^{125}I$ at all time periods studied. When rats were fed BHT in diets containing varying amounts of iodine (0.12, 0.15 or 0.3 mg iodine/kg feed) for 30 days, there was a significant ($p<0.001$) increase in thyroid weight in BHT-treated animals when compared to controls. BHT did not change levels of $T_3$ and $T_4$ 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 250 mg BHT/kg bw/day for 28 days showed an increase in the number of follicle cells (JECFA, 1996; Søndergaard and Olsen, 1982). Based on this observation the Panel noted that the NOAEL of this study would be 25 mg/kg bw/day.

Although rodents and humans share a common physiology in regard to the thyroid-pituitary feedback system, a number of factors contribute to the greater sensitivity of the rat to long-term perturbation of the pituitary thyroid axis which predisposes it to a higher incidence of proliferative lesions in response to chronic TSH stimulation than human thyroid.

Both humans and rodents have nonspecific low affinity protein carriers of thyroid hormone (e.g., albumin). However, in humans, other primates, and dogs there is a high affinity binding protein, thyroxine-binding globulin (TBG), which binds T4 (and T3 to a lesser degree); this protein is not present in rodents, birds, amphibians and fish, and has a 1000-fold greater binding affinity than nonspecific low affinity protein carriers.

Although qualitatively the rat is an indicator of a potential human thyroid cancer hazard, humans appear to be quantitatively less sensitive than rodents to developing cancer from perturbations in thyroid-pituitary status. Given that the rodent is a sensitive model for measuring the carcinogenic influences of TSH and that humans appear to be less responsive, effects on rodents would represent a conservative indicator of potential risk for humans. Rodent cancer studies typically include doses that lead to toxicity, including perturbation in thyroid-pituitary function over a lifetime. The relevance of the experimental conditions to anticipated human exposure scenarios (i.e., dose, frequency and time) should be considered. In addition, chemically-induced effects that are produced by short-term disruption in thyroid-pituitary function appear to be reversible, when the stimulus is removed.

***Other special studies***

A range of studies have been conducted which aim to illuminate basic biochemical and molecular biologic effects of BHT on various organs, mainly the liver. In the following section in vitro studies are described first, followed by studies with a single administration and finally brief descriptions of studies with repeated BHT administration:

BHT was studied in the concentration range of 0-50 µM in vitro in opsonised zymosan-stimulated neutrophils obtained from New Zealand White Rabbits. BHT showed concentration-dependent cytotoxicity, interaction with neutrophil membranes and reactive oxygen species (ROS) scavenger activity. It was pointed out by the study authors that BHT exerted a cytotoxic effect probably mediated by an interaction with neutrophil membranes and a scavenging of reactive oxygen species which could produce reactive intermediates (Kabeya et al., 2008; Saito et al., 2003).

The effect of BHT on cytochrome isoenzymes was investigated in cultured human hepatocytes. At the concentration range investigated (2-200 µM) a concentration-dependent increase in mRNA levels for CYP2B6 and CYP3A4 was demonstrated up to around 12- and 7-fold, respectively. BHT was not cytotoxic at these concentrations (Price et al., 2008).

Vanuyshin et al. (1998) observed that a single intraperitoneal injection of 60 mg BHT/kg bw results, within a few hours, in a strong increase in nuclear DNA(cytosine-5)-methyl transferase activity in the liver, kidneys, heart, spleen, brain and lungs of male rats.

Chevillard et al. (2010) found that both wild type and Nrf3 (NF-E2-related factor 3) deficient mice are sensitive to BHT. Female wildtype and Nrf3 deficient mice (10 weeks old) were treated once by gavage with BHT at a dose of 400 mg /kg bw. In the white adipose tissue the expression of genes implicated in adipogenesis were repressed following BHT exposure..

Allameh (1997) found that dietary exposure of rats to 0.75% BHT (estimated to be equivalent to approximately 375 mg BHT/kg bw) for two weeks induced liver glutathione S-transferase activity, whereas 0.05% BHT (estimated to be equivalent to approximately 25 mg BHT/kg bw/day) for 6 months did not. Similarly, Jang et al. (1999) found that 0.5% BHT in the feed of ICR mice (estimated to be equivalent to approximately 740 mg/kg bw/day) for 12 months induced the activity of intestinal and hepatic glutathione S-transferase.

Female rats were fed an antioxidant-supplemented diet containing 0.5% or 1.0% BHT (estimated to be equivalent to approximately 250 or 500 mg BHT/kg bw/day) with or without vitamin E acetate (0.4%) for 4 weeks, after which the liver and abdominal adipose tissue were analyzed for content of BHT and

α-tocopherol. The body weight gain was similar in all groups, independent of the diet, after the first week of treatment. At the end of the experiment the liver weights of the BHT-supplemented rats were increased compared to those of the control groups, and this difference was unaffected by vitamin E treatment. The liver concentration of α-tocopherol was decreased and inversely proportional to the BHT concentration in the diet. This attenuating effect of BHT on the hepatic α-tocopherol concentration was present both in animals with and without vitamin E supplementation. In contrast, BHT treatment did not alter the concentration of α-tocopherol in abdominal adipose tissue (Siman and Eriksson, 1996). This result is the opposite of what was found in the study of Frank et al. (2003), where an α-tocopherol sparing effect was found after 28 days of exposure to BHT. The main difference in design of these two studies is the gender of the rats.

BHT given orally to male rats for 7 consecutive days at dose levels of 75 or 450 mg/kg bw/day induced hepatocellular proliferation, an increase in hepatocyte apoptosis, and elevated immunoreactivity for transforming growth factor (TGF)-β1 in the liver during the treatment, and hepatocellular inhibition of mitosis following withdrawal (Furukawa et al., 2001).

7-Week-old male Sprague-Dawley rats were fed BHT in their diet for 28 days, corresponding to intakes in the range of 27.8-1158.8 mg/kg bw/day, prior to sampling for cDNA microarray analysis of hepatic RNA. BHT induced expression of 10 genes, including CYP2B1/2, CYP3A9, CYP2C6 involved in phase I metabolism and GST μ2 involved in phase II metabolism (Stierum et al., 2008).

In summary, BHT seems to be able to induce the activity of liver and pulmonary glutathione-transferase in mice, DNA methyl-transferase in several organs of rats, CYP3A-dependent testosterone 6-beta-hydroxylase, and the CYP2B and CYP3A forms of enzymes in rat and human. In addition, there are data which suggest that BHT represses genes implicated in adipogenesis. Conflicting results have been obtained with regard to apoptosis, liver cell proliferation and inhibition of mitosis in liver cells. Paradoxically, an α-tocopherol sparing effect in the liver has been observed in male rats, whereas in female rats an α-tocopherol attenuating effect has been observed.

## 4. Discussion

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel noted that the original studies on which previous evaluations were based were not all available for re-evaluation by the Panel.

BHT (E 321) is a synthetic antioxidant authorised as a food additive in the EU and was previously evaluated by the SCF in 1987 and by JECFA several times, the latest in 1996.

The SCF established an ADI of 0-0.05 mg/kg bw/day based on thyroid, reproduction and haematological effects in the rat. At its last evaluation JECFA allocated an ADI of 0-0.3 mg/kg bw/day for BHT, based on effects in the reproduction segments and hepatic enzyme induction seen in two separate 2-generation studies in rats. This study was probably not available at the time of the SCF evaluation.

Specifications have been defined in the EU legislation Directive 2008/48/EC and by JECFA (JECFA 2006). The purity is specified to be not less than 99%.

Absorption, distribution, metabolism and excretion of BHT have been studied in mice, rats, rabbits, chickens, monkeys and humans. Overall, these studies show that BHT is rapidly absorbed from the gastrointestinal tract. Upon absorption, BHT is distributed to the liver and body fat. Excretion is mainly via urine and faeces. The metabolism of BHT is complex. There may be important species differences. It is not known, for example, whether humans are capable of forming the quinone methides, metabolites that were found in rats and mice. In addition, biliary excretion in man seems to be less significant than in rats, rabbits and dogs.

The acute toxicity of BHT is low with oral $LD_{50}$ values of 1700-1970 mg BHT/kg bw in rats, 2100-3200 mg BHT/kg bw in rabbits, 10 700 mg BHT/kg bw in guinea pigs, 940-2100 mg BHT/kg bw in cats, and 2000 mg BHT/kg bw in mice.

In general, the genotoxicity studies on BHT are of limited design but the majority of evidence indicates a lack of potential for BHT to induce point mutations, chromosomal aberrations, or to interact with or damage DNA. The Panel recognised that positive genotoxicity results obtained with BHT in vitro may be due to pro-oxidative chemistry giving rise to formation of quinones and reactive oxygen species, and that such a mechanism of genotoxicity is generally considered to be subject to a threshold.

The Panel noted that the SCF established an ADI of 0-0.05 mg/kg bw based on thyroid, reproduction and haematological effects in the rat. However, a NOAEL of 25 mg BHT/kg bw/day was derived from a study in rats where electron microscopy of the thyroid glands of rats exposed to 500 mg BHT/kg bw/day for 28 days showed an increase in the number of follicle cells (JECFA, 1996; Søndergaard and Olsen, 1982).

The Panel has noted the discrepancy between the ADIs allocated by the SCF and JECFA. In 1987 the SCF reviewed all available studies on BHT, among them metabolic data from several species including man, mutagenicity studies, carcinogenicity studies in rats and mice, special studies on the thyroid, blood, and post-natal development and behaviour. Taking all these effects into account, the SCF considered that the likely NOAEL for BHT is approximately 100 mg/kg in the diet, equivalent to an intake of about 5 mg/kg bw/day. In the view of the nature of the effects, a safety margin of 100-fold was considered appropriate to establish an ADI of 0-0.05 mg/kg bw based on thyroid, reproduction and haematological effects in the rat (SCF, 1989).

Since the last SCF evaluation, two new 2-generation studies have been reported. One study has been published by Olsen et al. (1986). The other study is an unpublished report from The Robens Institute (Price, 1994) that was included in the JECFA evaluation published in 1996 and also submitted to EFSA after a public call for data.

The Panel considered that the effects of BHT on tumour formation reported in the Olsen et al. study (Olsen et al., 1986) are subject to a threshold since the genotoxicity studies generally indicate a lack of potential for BHT to induce point mutations, chromosomal aberrations, or to interact with or damage DNA. The BMD analysis performed by the Panel on the incidence of hepatocellular carcinoma in male rats induced by BHT as reported by Olsen et al. (1986) gave a $BMDL_{10}$ of 247 mg/kg bw/day.

JECFA concluded that the long-term study of Price (1994) gives a NOAEL of 25 mg/kg bw/day. Effects observed in the reproduction segments of the in utero/lifetime exposure studies (Olsen et al., 1986) were also taken into account in the JECFA derivation of the NOAEL. The JECFA applied a NOAEL of 25 mg/kg bw/day and an uncertainty factor of 100 to allocate an ADI of 0-0.3 mg/kg bw/day for BHT (JECFA, 1996).

The Panel agreed with the NOAEL of 25 mg/kg bw/day derived by JECFA from the study reported by Price (1994). The Panel concluded that the NOAEL in the Olsen et al. (1986) study was 25 mg/kg bw/day, based on effects on litter size, sex ratio and pup body weight gain during the lactation period, in the reproduction segment of the study.

Since the NOAEL of 25 mg/kg bw/day for the reproductive effects is below the $BMDL_{10}$ value of 247 mg/kg bw/day derived by the Panel based on the data of Olsen et al. (1986) for the incidence of hepatocellular carcinoma in male rats, the Panel concluded that this NOAEL of 25 mg/kg bw/day also covers the hepatocellular carcinoma observed in the long-term studies with BHT.

Overall, the Panel concluded that the present database justifies revision of the SCF ADI of 0-0.05 mg/kg bw/day. Based on the NOAEL of 25 mg/kg bw/day derived from both new 2-generation studies and an uncertainty factor of 100, the Panel established an ADI of 0.25 mg/kg bw for BHT.

Since JECFA's latest assessment in 1995, many mechanistic studies have appeared along with a long line of studies dealing with various interactions. These studies indicate that BHT may exert effects at doses far above the ADI, and some of these effects may be attributed to BHT working as a pro-oxidant at high concentrations, indirectly by inhibiting antioxidant defences through the depletion of non-protein thiols and by covalent modifications of protective enzymes (Sun et al., 2003). Various mechanisms have been suggested for the occurrence of lung and hepatic tumours in certain strains of mice and rats, and it seems that these effects may be due to many factors, such as inhibition of gap junctional intercellular communication (Guan et al., 1995; Trosko et al., 1990), increase in mitochondrial permeability, epigenetic changes due to induction of DNA methyl transferases (Vanyushin et al., 1998), induction of oxidative stress by the quinone methide metabolites (Faine et al., 2006), inhibition of antioxidant enzymes such as carbonyl reductase (Shearn et al., 2008).

Exposure estimates for children, adolescents and adults were made with the assumptions that the food category for which BHT is authorised can be found in liquid and solid food supplements, in fine bakery wares and in snacks, that the fat content of the latter food categories is 25% and that this amount of fat would contain BHT added at the maximum reported use level. This scenario would lead to average exposure estimates for BHT of 0.007-0.087 mg/kg bw/day, 0.005-0.043 mg/kg bw/day and 0.003-0.022 mg/kg bw/day for children, adolescents and adults, respectively. At the 95$^{th}$ percentile, exposure to BHT would be in the range of 0.04-0.296 mg/kg bw/day, 0.029-0.125 mg/kg bw/day and 0.07-0.161 mg/kg bw/day for children, adolescents and adults, respectively.

Unfortunately, very limited data are available for the consumption of chewing gum as a second food category for which the addition of BHT is authorised. Assuming a 10% extraction of BHT from chewing gum and selecting only those surveys with a sufficiently high number of consumers, the average exposure to BHT from chewing gum consumption is in the range of 0-0.003 mg/kg bw/day for children, 0-0.002 mg/kg bw/day for adolescents and 0-0.004 mg/kg bw/day for adults (consumers only). At the 95$^{th}$ percentile, exposure to BHT from chewing gum consumption is in the range of 0.004-0.0008 mg/kg bw/day for children, 0.003-0.006 mg/kg bw/day for adolescents and 0.004-0.008 mg/kg bw/day for adults.

Using a worst case scenario of combined exposure to BHT from food categories where its use as food additive is authorised, the Panel estimates potential exposure to be in the range of 0.01-0.03 mg/kg bw/day on average and 0.03-0.17 mg/kg bw/day at the 95$^{th}$ percentile for adults. For adolescents, combined exposure to BHT from all food categories would be on average in the range of 0.01-0.05 mg/kg bw/day and in the range of 0.04-0.13 mg/kg bw/day at the 95$^{th}$ percentile. For children, combined exposure to BHT from all food categories would be on average in the range of 0.01-0.09 mg/kg bw/day and in the range of 0.05-0.30 mg/kg bw/day at the 95$^{th}$ percentile.

Based on its use in food contact materials with a specific migration limit of 3 mg/kg food and with the assumption that every day throughout lifetime a person weighing 60 kg consumes 1 kg of food packed in plastics containing BHT, migration would increase exposure to BHT by 0.05 mg/kg bw/day. Assuming that children weighing 15 kg also consume 1 kg of food packed in plastics containing BHT in the maximum permitted quantity, exposure to BHT of children would be increased by 0.2 mg/kg bw/day.

BHT is also used in cosmetics at concentration of 0.0002-0.5%; in pharmaceutical preparations, in ointments, preparations for medical bathing solutions, and similar products which are intended for topical application. Some absorption of BHT into the skin has been reported, but BHT appears to pass only slowly through the skin the majority remaining on the surface. Consequently dermal application does not produce substantial systemic exposure to BHT or its metabolites (CIR, 2002).

The Panel noted that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 5 mg/kg.

## CONCLUSIONS

The Panel concluded that the present database justifies the revision of the ADI of 0-0.05 mg/kg bw/day set by the SCF.

Based on a NOAEL of 25 mg/kg bw/day from two 2-generation studies in rats and an uncertainty factor of 100, the Panel derived an ADI of 0.25 mg/kg bw/day.

Since the NOAEL of 25 mg/kg bw/day for the reproductive effects is below the $BMDL_{10}$ values for hepatocellular carcinomas in long-term studies with BHT, the Panel concluded that this NOAEL also covers this effect.

The Panel noted that exposure of adults to BHT from its use as food additive is unlikely to exceed the newly derived ADI of 0.25 mg/kg bw/day at the mean and for the high consumers ($95^{th}$ percentile). Exposure of children to BHT from its use as food additive is also unlikely to exceed this ADI at the mean, but is exceeded for some European countries (Finland, The Netherlands) at the $95^{th}$ percentile. If exposure to BHT from its use as food contact material is also taken into account the new ADI would be exceeded by children at the mean and at the $95^{th}$ percentile,

The Panel noted that the JECFA specification for lead is $\leq$ 2 mg/kg whereas the EC specification is $\leq$ 5 mg/kg.

## DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the DHI. September 2010.

2. FoodDrinkEurope. Contribution to EFSA's call for data on E 321 and E 320. October 2011.

3. Lanxess Deutschland GmbH. Reply to EFSA "Call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants" (published: 23 November 2009) on 16 April 2010.

4. Oxiris Chemicals S. A., Spain. Reply to EFSA "Call for scientific data on food additives permitted in the EU and belonging to the functional classes of preservatives and antioxidants" (published: 23 November 2009) on 25 June 2010.

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## GLOSSARY AND/OR ABBREVIATIONS

| | |
|---|---|
| ADI | Acceptable Daily Intake |
| AHF | Altered Hepatocellular Foci |
| ALBA | Dutch database of food additives |
| ANS | Scientific Panel on Food Additives and Nutrient Sources added to Food |
| BHA | Butylated hydroxyanisole |
| BHT | Butylated hydroxytoluene |
| BHTOH | 6-t-butyl-2-(hydroxy-t-butyl)4-methylphenol |
| BHTBzOH | 2,6-di-t-butyl-4-hydroxymethylphenol |
| BHT-QM | BHT - quinone methide (2,6-di-tert-butyl-4-methylene-2,5-cyclohexadien-1-one) |
| BMD | Benchmark Dose |
| BMDL | Benchmark Dose Level |
| BPPOH | 2-tert-butyl-6-(1'-hydroxy-1'-methyl)ethyl-4-methylphenol |
| BSO | Buthionine sulfoximine |
| BUN | Blood urea nitrogen |
| CAS | Chemical Abstracts Service |
| DBP | 2,6-di-tert-butyl phenol |
| DMBA | 7,12-dimethylbenz(a)anthracene |
| DNA | Deoxyribonucleic Acid |
| EFSA | European Food Safety Authority |
| EC | European Commission |
| $ED_{50}$ | Effective Dose, 50% |
| EU | European Union |
| FCS | Food Nomenclature |
| FID | Flame ionization detector |
| FSA | Food Standards Agency |
| FTIR | Fourier transform infrared |
| GC-MS | Gas-chromatography coupled with mass spectrometry |
| GGT | Gamma-glutamyl trans-peptidase |
| GJIC | Gap junctional intercellular communication |
| GOT | Glutamic-oxaloacetic transaminase |
| GPT | Glutamic-pyruvic transaminase |
| GSH | Glutathione |
| GST | Glutathione-S-transferase |
| HBHT | 2,6-di-tert-butyl-4-hydroperoxy-4-methylcyclohexa-2,5-dien-1-one |

| | |
|---|---|
| HPLC | High-performance liquid chromatography |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| IARC | International Agency for Research on Cancer |
| $LD_{50}$ | Lethal Dose, 50% i.e. dose that causes death among 50% of treated animals |
| MHCD | 2,6-di-tert-butyl-4-hydroxy-4-methylcyclohexa-2,5-dione |
| MLA | Mouse lymphoma assay |
| MN | Mouse micronucleus |
| MNRETs | Micronucleated reticulocytes |
| MPL | Maximum Permitted Level |
| NLCS | Netherlands Cohort Study |
| NOAEL | No-Observed-Adverse-Effect Level |
| PROD | Pentoxyresorufin-O-depentylase |
| ROS | Reactive Oxygen Species |
| SCF | Scientific Committee on Food |
| SCOOP | A scientific cooperation (SCOOP) task involves coordination amongst Member States to provide pooled data from across the EU on particular issues of concern regarding food safety |
| SOD | Superoxide dismutase |
| TBG | Thyroxine-binding globulin |
| TBHQ | tert-butylhydroquinone |
| TGF | Transforming Growth Factor |
| TSH | Thyroid-Stimulating Hormone |
| OECD | Organisation for Economic Co-operation and Development |