2,6-Di-*tert*-butyl-hydroxytoluene and its metabolites in foods

Bárbara Nieva-Echevarría, María J. Manzanos, Encarnación Goicoechea, María D. Guillén*

Food Technology, Faculty of Pharmacy, Lascaray Research Center, University of the Basque Country (UPV/EHU). Paseo de la Universidad nº 7, 01006 Vitoria, Spain

*Telf: 34-945-013081. E-mail: mariadolores.guillen@ehu.es

Running Head: **BHT and metabolites in foods**

Abstract

2,6-Di-*tert*-butyl-hydroxytoluene (BHT, E-321) is a synthetic phenolic antioxidant which has been widely used as an additive in the food, cosmetic, and plastic industries for the last 70 years. Although it is considered safe for human health at authorized levels, its ubiquitous presence and the controversial toxicological data reported are of great concern for consumers. In recent years, special attention has been paid to these 14 metabolites or degradation products: BHT-CH_2OH, BHT-CHO, BHT-COOH, BHT-Q, BHT-QM, DBP, BHT-OH, BHT-OOH, TBP, BHQ, BHT-OH(t), BHT-OH(t)QM, 2-BHT, and 2-BHT-QM. These derived compounds could pose a human health risk from a food safety point of view, but they have been little studied. In this context, this review deals with the occurrence, origin, and fate of BHT in foodstuffs, its biotransformation into metabolites, their toxicological implications, their antioxidant and prooxidant properties, the analytical determination of metabolites in foods, and human dietary exposure. Moreover, noncontrolled additional sources of exposure to BHT and its metabolites are highlighted. These include their carry-over from feed to fish, poultry and eggs, their presence in smoke flavorings, their migration from plastic pipelines and packaging to water and food, and their presence in natural environments, from which they can reach the food chain.

KEYWORDS: BHT, metabolites, food safety, dietary exposure, analytical techniques.

List of Chapters

1. Introduction

Synthetic phenolic antioxidants were originally developed for petroleum protection and in the late 1940s began their use in the food industry to delay lipid oxidation, and thus to increase food shelf-life (Maziero and others 2001). The phenolic antioxidants currently allowed as food additives by the European authorities are these 9 substances: *alpha*-, *gamma*-, and *delta*-tocopherol, propyl-, octyl- and dodecyl-gallate, *tert*-butyl-hydroquinone (TBHQ), 2-*tert*-butyl-4-hydroxyanisole (BHA), and 2,6-di-*tert*-butyl-hydroxytoluene (BHT).

In the last years, it is being invoked the need of further research about the dual antioxidant-prooxidant role of many compounds in relation to their effects on human health and also on foods (Aruoma 1991; Frankel 2007; Halliwell 2011). In addition, consumers are currently concerned about the use of certain food additives due to their potential toxicity. As a result, additive-free labeling is a well-known trend in the food industry. In this sense, the widespread use of BHT as an antioxidant can be considered a matter of public health concern, because of the potential toxicological effects attributed to BHT itself and to metabolites derived from it (Branen 1975; Babich 1982; Malkinson 1983; Kahl 1984; Bomhard and others 1992; Williams and others 1999). **Table 1** summarizes some of the main BHT metabolites found in different matrixes, coming either from metabolic transformations or from degradative processes occurring in foods, plastics, or the environment. Special attention should be paid to these derived compounds, since the toxic effects of BHT are thought to be caused by its metabolites rather than by the parent compound (Thompson and Trush 1986).

In this context, and taking into account the multiple sources of exposure to BHT and its metabolites, this review deals with its occurrence and origin, their possible dual role

as antioxidants and prooxidants, the fate of BHT in foodstuffs, its biotransformation into metabolites, their toxicological implications, some dietary BHT exposure studies and the established limits, additional sources of exposure, and the analytical determination of BHT metabolites in foods.

2. Occurrence of BHT and its origin

BHT is one of the most commonly employed food antioxidants and its use in Europe is restricted to different dosages (EU Directive No 95/2/EC, EU Regulation (EC) No 1333/2008). It can be added singly or in combination with gallates, BHA, or TBHQ in amounts of up to 100 mg/kg in fats and oils for the professional manufacture of heat-treated foods, in frying oil and frying fat (excluding olive pomace oil), in lard, in fish oil, and in beef, poultry, and sheep fats. BHT can also be added to seasonings and condiments, either individually or in combination with gallates, BHA, and TBHQ, in amounts up to 200 mg/kg. Furthermore, BHT is also authorized in chewing-gum and other food supplements at maximum concentrations of 400 mg/kg, either alone or in combination with gallates and BHA. It must be noted that although maximum permitted levels (MPLs) of BHT are well established in certain food products, like those shown in **Table 2**, its presence in other foods is also possible, either because it is present in any of the ingredients or for other reasons.

In addition to its use as a food antioxidant, BHT may also be added to animal feeds, food packaging materials, pharmaceuticals, pesticides, rubbers, plastic pipelines, biodiesel fuel, lubricants, paints and inks, personal care products, and cosmetics as a stabilizer or anti-skinning agent. Due to the presence of BHT in the waste waters

generated by these industries, it can also be found in natural environments like soil, water, and air. BHT can also reach the food chain by these means.

Although BHT is a well-known synthetic compound, it must be noted that a natural origin for it is also possible. It has been evidenced that this molecule can be endogenously generated by some freshwater phytoplankton submitted to oxidative stress conditions (Babu and Wu 2008). Its presence in oak wood and in the pericarp of litchi fruit has also been reported (Guillén and Manzanos 2001; Jiang and others 2013).

3. BHT and its metabolites as antioxidants or prooxidants

One of the main reasons for food deterioration during processing or storage is lipid oxidation, which evolves through different steps and gives rise to the formation of very reactive compounds. It must be noted that this degradation process occurs not only in food lipids, but also in those present in cells and biological systems.

Antioxidant properties of BHT rely on its molecular configuration, because, like other synthetic phenolic antioxidants, it possesses a labile hydrogen atom in the hydroxy group that can be donated and reduce the free radicals generated at the initiation step of lipid oxidation. Thus, BHT itself is oxidized and the subsequent derived radical is stabilized by electronic delocalization in the benzene ring. This way, BHT can stop radical oxidation propagation, which retards lipid oxidation and extends food shelf-life (Warner and others 1986a). BHT has been reported to efficiently react against strong oxidizing radicals such as singlet oxygen ($O_2(^1\Delta_g)$), hydroxyl radicals ($^{\cdot}OH$), and peroxyl radicals ($^{\cdot}OOR$), although the mechanism of quenching reactions may vary, depending on the reactive oxygen species involved and the local environment (Lambert and others 1996). The

121 reaction pathways previously proposed for BHT antioxidant activity under experimental
122 conditions are summarized in **Figure 1**. Nevertheless, the antioxidant activity of this
123 molecule at high temperatures, such as frying conditions, can greatly differ from that
124 developed at moderate or low temperatures, as during storage. Several authors reported
125 rapid loss of BHT at high temperatures (> 140 ºC) and noticed lower antioxidant activity
126 (Zhang and others 2004; Marmesat and others 2010). This fact was attributed not only to
127 the compound volatilization, but also to its transformation into metabolites. These results
128 are in agreement with the lower antioxidant activity observed at 70 ºC for BHT-OOH,
129 BHT-CHO, and BHT-Q than for the parent compound BHT (Fujisawa and others 2004).

130 However, BHT has also been reported to exert **prooxidant** effects under certain
131 conditions. Thus, when BHT was added in excess to a wheat seedling medium in aerobic
132 conditions, an enhancement of the generation rate of superoxide anion was observed. This
133 is a reactive particle that may damage cellular structures at high concentrations (Smirnova
134 and others 2002). In addition, an increase in hepatic microsomal lipid peroxidation was
135 observed in rats fed with diets containing 0.2% of BHT for 30 days (Yamamoto and others
136 1995). Due to this ability of BHT to exert prooxidant effects at high concentrations, it has
137 been used to induce experimental models of oxidative stress in several animals and fungi
138 in order to study the protective effects of other compounds (Faine and others 2006; Lin
139 and others 2007; Nanou and Roukas 2010; Awah and others 2012).

140 Although the above-mentioned studies suggest a prooxidant activity, there is limited
141 information available concerning the conditions and the mechanisms under which BHT
142 exerts a prooxidant behavior. Some authors have reported that at high aeration rate, BHT
143 can react with molecular oxygen rather than with the reactive oxygen species present,
144 yielding BHT-phenoxyl radical and superoxide anion (Smirnova and others 2002, Nanou

145 and others 2010). The reaction pathways proposed for BHT prooxidant activity are
146 summarized in Figure 1. In addition, the phenolic radical itself may undergo redox
147 recycling which can be a critical factor depending on the reductant involved (Kagan and
148 others 1990). However, it has to be noted that BHT-phenoxyl radical has been reported
149 to be relatively stable (Lambert and others 1996).

150 Furthermore, the potential reactivity of BHT-derived metabolites should be taken into
151 account; some studies reported that not only BHT but also its metabolites, such as BHT-
152 Q and BHT-QM, can act as prooxidants (Nagai and others 1993; Bolton and others 1990;
153 Guyton and others 1991); this could help to explain the toxicological effects attributed to
154 some of the latter, further commented on chapter 6. Special efforts should be made to
155 fully understand the antioxidant-prooxidant activity of BHT, the reaction pathways
156 undergone, the necessary conditions for this to take place, and the potential reactivity of
157 all the metabolites generated as a result of BHT activity, in order to avoid undesired
158 reactions, either in foods or in biological systems.

159

160 ## 4. Fate of BHT in foods

161 During food processing and storage the evolution of lipid oxidation and, consequently,
162 the evolution of the antioxidant molecules originally present, can be very different
163 depending on the temperature at which the process takes place. Thus, taking into account
164 the dual role (antioxidant-prooxidant) previously mentioned, knowledge concerning the
165 effect of temperature on BHT transformation is of paramount importance. As far as we
166 know, there is a lack of studies dealing with this topic at low or moderate temperatures.
167 Only a few studies have addressed the fate of BHT in foods during **processing at high**
168 **temperatures**. In fact, it has been shown by several authors that under frying conditions

the loss of antioxidants, either natural or synthetic, is very fast and their activity very low. As mentioned before, their low effectiveness at high temperatures is attributed not only to their volatilization and steam distillation caused by the water boiled out of the cooked food, but also to their rapid degradation (Augustin and Berry 1983; Tsaknis and others 2002; Allam and Mohamed 2002; Zhang and others 2004).

Regarding **volatilization** at high temperatures, Warner and others (1986b) concluded that BHT showed the highest volatilization, in comparison with BHA and TBHQ, reaching 28% during cookie baking at 195 ºC for 10 min and 49% after submitting potatoes to deep-frying in lard at 190-200 ºC for 1 h. Similar results were obtained by Santos and others (2012) who submitted pure propyl-gallate, BHT, TBHQ, and BHA to thermal stability evaluations at 110 ºC and concluded that BHT volatilized within the first few hours of heating, showing the lowest thermal resistance. Hamama and Nawar (1991) submitted the same pure synthethic antioxidants to 185 ºC for 1 h and observed that BHT showed the highest volatility, because one-fourth of the original amount was lost by evaporation. This is in agreement with the lower boiling point of BHT (264 ºC) in comparison with those of BHA (268 ºC) and TBHQ (291 ºC).

Concerning the **transformation** of BHT at high temperatures, there are only a few studies on the detection, isolation, and identification of its degradation products in food matrices. Under these conditions BHT reacts directly with oxygen giving rise to BHT-OOH as the main oxidation product (Kharasch and Joshi 1957), which is unstable and, in turn, could generate other derived products. It must be noted that when pure BHT-OOH was submitted to thermolysis at high temperatures (185-210 ºC), the formation of BHT-CHO, BHT-OH, and BHT-Q was reported (Warner and others 1986a). Another study gave information about the degradation products of pure BHT submitted to 185 ºC for up

to 1 h, obtained by gas chromatography/mass spectrometry (GC/MS) (Hamama and Nawar 1991); the formation of 5 compounds was reported and their possible generation pathways proposed. Among the derived compounds generated, 5-methyl-7-*tert*-butyl-2,2-dimethyl-2,3-dihydrobenzo(b)furan, 2,6-bis(1,1-dimethylethyl)-4-methyl-1-methoxybenzene, TBP, and 2 dimeric derivatives, one of them being 2-BHT, were tentatively identified by their mass spectra. It has to be noted that the spectra given by these authors to the 2 first compounds are very similar to those of BHT-QM and BHT-CHO (Fries and Püttmann 2002; Goicoechea and others 2008; Hernández and others 2009). The dimer 2-BHT has also been detected in water (Fries and Püttmann 2002). The oxidation of this latter dimer gives rise to a stilbenequinone derivative, called 2-BHT-QM, whose formation was reported in vegetable oils containing BHT heated at 190 ºC for 11 days (Leventhal and others 1976).

It is remarkable that the compounds generated in the degradation of BHT at high temperatures depend on the food nature in which it is contained and on the processing conditions. Thus, during cookie baking no transformation of the added BHT was detected, whereas during deep-frying its chemical alteration was clearly evident, only 14% of the added BHT remaining unaffected in the frying medium (lard) (Warner and others 1986b). In this context and taking into account the lack of sound studies on the toxicological effects of BHT-derived compounds, the addition of BHT to frying oils could be questioned. From a food safety point of view, further research is needed regarding the nature and the extent of BHT transformation products that can be formed in foodstuffs when submitted to different processes, either at high or at low temperatures like storage, as well as concerning the BHT degradation pathways that generate them. In fact, it has to be noted that a wide variety of products which are consumed daily and which may contain BHT are submitted to thermal processing. These include bread, cereals, pastry, cakes,

biscuits, cookies, sweets, chips, and snacks. Others, such as oils, fats, and salad dressings, are submitted to long-term storage.

In the food industry it is a common practice to add BHT, and other synthetic phenolic antioxidants as a mixture, in order to increase anti-oxidation effectiveness. However, it has been reported that the concomitant administration of BHT and BHA in mice enhanced the formation of BHT-QM and 2-BHT-QM, and the covalent binding of BHT to microsomal proteins in lungs, with detrimental toxic effects (Thompson and Trush 1986). It is remarkable that the generation of these 2 metabolites was not observed in the absence of BHA. Since the addition of mixtures of synthetic phenolic antioxidants is usual in the food industry, further research is needed on this topic.

5. Biotransformation of BHT

BHT undergoes biotransformational processes not only in foods, natural environments, such as air, dust, soils, or water (Fries and Püttmann 2002, 2004; Fernandez-Alvarez and others 2009; Rodil and others 2010, 2012), but also in living organisms. The metabolism of BHT is very complex and it has been investigated in different animal species, such as rats (Daniel and Gage 1965; Ladomery and others 1967; Daniel and others 1968; Holder and others 1970a; Shaw and Chen 1972; Matsuo and others 1984; Conning and Phillips 1986), mice (Matsuo and others 1984), rabbits (Dacre 1961; Conning and Phillips 1986), chickens (Frawley and others 1965), monkeys (Allen 1976; Conning and Phillips 1986), dogs (Gage 1966), and also in humans (Daniel and others 1967, 1968; Holder and others 1970b; Conning and Phillips 1986). As BHT undergoes several reactions during biotransformation, a large number of intermediate

241 metabolites have been identified. However, their nature and concentration depend on the
242 environmental conditions and on the animal species.

243 Although the changes undergone by BHT during *in vivo* **digestion** processes have not
244 been studied, after submission of a fluid deep-frying fat containing BHT and BHT-QM
245 to an *in vitro* gastrointestinal digestion model, both these were detected in the digested
246 samples (Goicoechea and others 2008). These results indicate that BHT and its toxic
247 metabolite could remain bioaccessible for intestinal absorption. Due to the great
248 importance of the matter, further research on the potential oxidation process of BHT
249 during digestion and on the nature of the metabolites arising from it will be needed.

250 A rapid **absorption** of this compound from the gastrointestinal tract and subsequent
251 **distribution** to the liver and body fat has been observed (Conning and Phillips 1986).
252 Some studies referring to the rate of distribution of BHT in different organs after ingestion
253 have been carried out. The distribution of this compound in stomach, intestines, gall
254 bladder, urinary bladder, liver, kidney, spleen, and salivary gland has been proved in mice
255 after ingestion of 20 mg/ kg body weight (bw) of BHT. The highest concentration was
256 found in liver, kidney, and blood 3 h after the treatment (Matsuo and others, 1984).
257 Likewise, in humans a single oral dose of 0.5 mg/ kg bw provoked gradually increasing
258 levels in plasma until 75 min after intake (Verhagen and others 1989); however large
259 variations (from not detectable to 250 ng/ml) were observed among the subjects under
260 study.

261 Studies concerning BHT **metabolism** have shown that, unlike other synthetic
262 antioxidants, BHT is a potent inducer of the microsomal monooxygenase system and its
263 major route of degradation is oxidation catalyzed by cytochrome P450 (Conning and
264 Phillips 1986). As can be observed in **Figure 2**, its metabolism takes place through 2 main

pathways: the oxidation of the alkyl substituents and of the benzene ring. Regarding the **oxidation of alkyl substituents**, BHT can show oxidation in the *p*-methyl group and in one or both of the *tert*-butyl groups; however, neither process is mutually exclusive (Daniel and others 1968). Metabolic differences among species have been reported: whereas oxidation of the *p*-methyl group is the major metabolic route for rats, rabbits, and monkeys, the oxidation of *tert*-butyl groups predominates in humans and mice (Conning and Phillips 1986). When **oxidation of the *p*-methyl group** occurs, BHT-COOH is considered to be the main metabolite, and it may be generated via the corresponding aldehyde (BHT-CHO) and alcohol (BHT-CH_2OH) (Matsuo and others 1984); oxidation of BHT-CHO can also give rise to BHT-Q (Oikawa and others 1998). In addition, further metabolism of BHT-COOH in rat liver could provoke decarboxylation to DBP, followed by the formation of BHQ and BHT-Q (Yamamoto and others 1991). When the **oxidation of the *tert*-butyl groups** takes place, BHT-OH(t) and its derivative BHT-OH(t)QM can be formed (Thompson and others 2001). As far as the second route of **oxidation of the aromatic ring** of BHT is concerned, this can lead to the formation, among others, of BHT-Q and of BHT-OOH; in turn, the latter can generate BHT-QM (Becker 1969; Guyton and others 1991). Regarding this latter metabolite, it is noteworthy that there is no evidence yet about its generation in humans (EFSA 2012); however, it could be absorbed through the diet (Goicoechea and others 2008).

As far as the **excretion** of BHT and its metabolites is concerned, in man they are excreted mainly in the urine, whereas in rodents 50-80% is eliminated in the feces (Daniel and others 1967; Daniel 1986). When a single oral dose of 20 mg/ kg bw of BHT was given to mice, approximately 98% of the BHT was excreted after 7 days. When the same low oral dose was given for 10 consecutive days, its half-life in liver, kidney, lung, testis, and blood ranged from 5 to 15 days (Matsuo and others 1984). In humans, 50% of BHT

was excreted within the first 24 h after a single oral dose of 40 mg/ kg bw (Daniel and others 1967). The rest was slowly excreted over the next 10 days, suggesting higher tissue retention in humans than in rats. The major metabolites indentified in human urine after a single dose of 1 g of BHT were BHT-COOH and its ester glucuronide (Holder and others 1970b). After feeding rats and mice with radio-labeled BHT more than 43 metabolites were identified in the urine and feces, the main ones also being BHT-COOH and its ester glucuronide (Matsuo and others 1984).

Some studies which focus on the **accumulation** of BHT in fatty tissues after feeding animals with diets containing BHT can also be found. Frawley and others (1965) fed chickens with a diet containing 200 mg/kg of BHT for 10 weeks and reported that their edible portions contained between 1 and 3 mg/kg of BHT and metabolites; slightly higher concentrations were found in isolated fat, skin, liver, and viscera. Eggs produced by hens fed similar diets contained approximately 2 mg/kg of BHT and metabolites. Similar results were obtained by Daniel and Gage (1965) who reported low bioaccumulation in rats fed diet with 500 mg/kg of BHT for 35 days and rapid elimination of retained compounds when rats returned to their normal diet. Gender differences were also observed, 30 mg/kg of BHT being detected in male rat fat and 45 mg/kg in that of female rats. Matsuo and others (1984) did not observe any tendency to accumulate BHT in major tissues in mice. More recently, Holaas and others (2008) studied the liver deposition and toxicological effects in Atlantic salmon (*Salmo salar*) fed graded levels of BHT for 12 weeks, followed by a 2-week depuration period. They concluded that BHT was highly retained in fish liver, as only 8-13% of fed BHT was eliminated during the depuration period. It is remarkable that this is just a part of the total concentration in the whole fish, since BHT may have been distributed and accumulated in other organs. The authors pointed out that this high accumulation of BHT or its metabolites may have potential toxic

315 health effects for both fish and fish consumers through carry-over processes from the fish
316 products.

317 With regard to accumulation in humans, it was concluded that on a dose/ bw basis,
318 BHT reaches higher concentrations in the fatty tissues of humans than in those of rats
319 (Conacher and others 1986). It has been estimated that the bioconcentration factor of BHT
320 in human adipose tissue is around 45 times higher than that calculated for rats (Geyer and
321 others 1986). In 1970 the concentration of BHT found in the body fat of United Kingdom
322 (UK) and United States of America (USA) residents was 0.23 and 1.30 mg/kg,
323 respectively (Collings and Sharratt 1970). In 1986 in Canada, a mean level of 0.12 mg/kg
324 of BHT was reported in human adipose tissue (Conacher and others 1986). No BHT was
325 found in human plasma during an Italian study, although detectable levels of BHT (0.003
326 mg/kg) were found in the peritoneum of 64% of the subjects analyzed (Bianchi and others
327 1997). More recently, the presence of BHT and its metabolite BHT-CHO was reported in
328 the breast adipose tissue of women with breast cancer (Hernández and others 2009).

329

330 **6. Toxicological implications of BHT and its metabolites**

331 Due to the widespread use of BHT in foods and in many other products in the last 70
332 years, and to the consequent human long-term exposure, a great number of toxicity studies
333 have been carried out in order to assess its safety. These have been reviewed by some
334 authors (Branen 1975; Babich 1982; Malkinson 1983; Kahl 1984; EFSA 2012). The fact
335 that most of these studies were accomplished using several animal species makes it
336 difficult to extrapolate them to humans, because the metabolites generated could be
337 different and the conditions of exposure may not mimic those occurring in humans. In
338 this context, controversial results regarding the toxicity of BHT and its metabolites can

be found; data reported in toxicological studies have attributed to BHT a wide variety of effects on laboratory animals, either beneficial, deleterious, or none.

When it comes to the **potential beneficial effects** of BHT, it has been reported that BHT is able to inactivate *in vitro* lipid-containing mammalian and bacterial viruses (Snipes and others 1975), to protect chickens exposed to Newcastle disease virus (Brugh 1977) and rabbits against artherosclerosis (Björkhem and others 1991), to inhibit skin tumor promotion in rodents (Slaga 1995), to protect turkeys against aflatoxin B1 (Klein and others 2003), and to decrease total cholesterol levels in plasma and liver in monkeys (Branen and others 1973).

With regard to humans, there is an evident lack of studies which relate BHT intake to disease. A prospective cohort study carried out in The Netherlands found no association between the consumption of mayonnaise and creamy salad dressings with BHT and stomach cancer risk. Even a statistically nonsignificant decrease in stomach cancer risk was observed with increasing BHT intake (Botterweck and others 2000). However, it must be noted that the intake of other food products containing BHT was not considered. Therefore, further studies on this topic would be of a great interest.

In contrast with the above-mentioned potential protective effects in animals, many other studies have reported **potential toxicity** derived from the ingestion or administration of BHT. As for **acute oral toxicity**, although this is considered low in animals (JECFA 1996), it must be noted that two clinical cases were reported in patients who suffered acute neurotoxicity and gastritis after ingesting a high dose of BHT (4 and 80 g without medical prescription) to cure recurrent genital herpes (Grogan 1986; Shlian and Goldstone 1986).

Regarding **short-term subchronic toxicity** studies, it has been reported that BHT causes dose-related increase in the incidence and severity of toxic nephrosis in mice (Takahashi 1992), nephrotoxicity and pneumotoxicity in rats (Al-Akid and others 2001), and in chicken a marked congestion of the liver and kidney, as well as diffuse enlargement of the liver with rounded borders and rupture with hemorrhaging (Rao and others 2000). It has to be noted that the EFSA Panel (2012) pointed out certain inconsistencies in the findings obtained from the short-term and subchronic toxicity studies.

The **genotoxicity** studies on BHT reviewed by Bomhard and others (1992) and by Williams and others (1999) concluded that BHT does not represent a genotoxic risk, because most of the studies carried out to that date had shown BHT was not able to induce mutations or to damage deoxyribonucleic acid (DNA). Nevertheless, it must be mentioned that other studies reported contrary results. Thus, Nagai and others (1993) investigated the effect of BHT and 7 of its metabolites on *in vitro* DNA cleavage and reported the strongest effect for BHT-Q at the lowest concentration. The ability to cause DNA cleavage has also been attributed to BHT-CHO and BHT-OOH, whereas such effect was not observed for BHT, BHT-CH_2OH, BHT-COOH, and BHT-QM. These results are in agreement with those of Oikawa and others (1998) who studied *in vitr*o the mechanism of DNA damage by BHT-OOH and BHT-Q, and concluded that the former participates in oxidative DNA damage directly, whereas the latter causes it through H_2O_2 generation, which leads to internucleosomal fragmentation. Therefore, carcinogenesis risk and cell apoptosis would be dependent on the intensity of the damage and the ability of the cell to repair it. The Panel on Food Additives and Nutrient Sources Added to Food of the European Food Safety Authority (EFSA) recognized that these positive genotoxicity results may be due to the prooxidative chemistry of BHT, which gives rise to reactive metabolites (Aruoma 1991; EFSA 2012).

Some studies addressed the **carcinogenicity** and **chronic toxicity** of BHT and its metabolites in rodents with contradictory results. Thus, mice fed dietary BHT for a year developed marked hyperplasia of the hepatic bile ducts with an associated subacute cholangitis (Clapp and others 1973). Moreover, after 104 weeks of administration of BHT, the formation of hepatocellular tumors in male mice was observed (Inai and others 1988). After 10 months of feeding mice with a diet containing different amounts of BHT, an increased incidence of liver tumors in male, but not female, animals was also reported (Lindenschmidt and others 1986). However, in a similar study no evidence of the carcinogenicity of BHT administered to mice was observed (Shirai and others 1982). Studies performed in rats also reported dose-related increases in hepatocellular adenomas and carcinomas (Olsen and others 1986); nevertheless, other studies carried out with rats showed no consistent carcinogenic effects (Hirose and others 1981; Williams and others 1990).

In 1987 the International Agency for Research on Cancer evaluated BHT and classified it in group 3 of carcinogens (*Not classifiable as to its carcinogenicity to human*s), along with other substances like certain polycyclic aromatic hydrocarbon (PAH) compounds. In those days there was limited evidence of its carcinogenicity in experimental animals and no evaluation could be made regarding humans because no data were available (IARC 1987). On the other hand, several studies have demonstrated the potential of BHT to act either as a tumor promotor or as a tumor suppressor, modulating the carcinogenicity of some well-known carcinogens (Williams and others 1986; 1996; Malkinson and others 1997; Sun and others 2003). In this context, further elucidation of the role of BHT and its metabolites in the development of tumors is required.

Additional effects such as behavioral changes, reduction in body weight gain and decrement in body weight have also been observed after long-term administration of BHT to mice and rats (Stokes and Scudder 1974; Olsen 1986; Tanaka and others 1993; Price 1994; JECFA 1996).

Although, like many other xenobiotic compounds, the toxic effects may be attributed more to BHT metabolites than to their parent compound, only a few studies have focused on their carcinogenicity and toxicity, and not only on that of BHT. Thus, BHT-QM is a very reactive compound which is considered to play a significant role in hepatoxicity, pneumotoxity, and skin tumor promotion in mice (Mizutani and others 1987; Bolton and others 1990; Guyton and others 1991; Chevillard and others 2010). In addition, it was reported that another quinone derivative, BHT-OH(t)QM, is chemically more reactive than BHT-QM, and it has been recognized as the principal metabolite responsible for lung tumor promotion activity of BHT in mice (Thompson and others 2001; Kupfer and others 2002). Due to their high electrophility, quinone methide derivatives form adducts with several proteins, including enzymes that protect cells from oxidative stress; this prooxidant state can also lead to cell oxidative damage (Lemercier and others 2004; Meier and others 2007; Shearn and others 2011). It must be noted that relationships between chronic oxidative stress and tumor promotion are well known (Philip and others 2004).

7. Dietary exposure studies and established limits

Risk assessments of food additives rely on the available toxicological data in order to define their acceptable dietary intake (ADI). In 1987 the EU Scientific Committee for Food (SCF) established an ADI for BHT of 0-0.05 mg/kg bw/day based on thyroid, reproduction, and hematological effects observed in a 90-day feeding study with rats

(Commission of the EC 1989). Eight years later, in 1995, the Joint FAO/WHO Expert Committee on Food Additives allocated a 6 times higher ADI for BHT, 0-0.30 mg/kg bw/day, on the basis of hepatic enzyme induction in a 22-month feeding study with rats (JECFA 1996). Seventeen years later, in 2012, the Panel on Food Additives and Nutrient Sources Added to Food of EFSA established an ADI of 0-0.25 mg/kg bw/day (EFSA 2012), based on an uncertainty factor (safety margin) of 100 and two 2-generation studies with rats that revealed a no observed adverse effect level (NOAEL) of 25 mg/kg bw/day for BHT (Olsen and others 1986; Price 1994). The panel concluded that this NOAEL also covers the hepatocellular carcinomas observed in long-term studies on BHT.

Table 2 summarizes the studies carried out in different countries to evaluate BHT dietary intake, expressed either as estimated daily intake (EDI) or as theoretical maximum daily intake (TMDI). The aim of these studies was to monitor the exposure of their population to BHT and to evaluate the risk of exceeding the established ADI. However, since the selection of food categories containing BHT and the methodological approaches are very different, direct comparison of the results of the different studies should be made with caution. In most of them, the estimates were based on the distribution of food intakes observed in dietary surveys (individual, household, or market surveys), assuming BHT to be present at MPLs in all foods in which it is authorized. Other less common options were the use of concentration data of BHT in foods provided by the food industry (EFSA 2012) or analytically determined (Soubra and others 2006), the latter being considered the most accurate way to estimate the intake. Nevertheless, it has to be added that the selection of the food categories, the sampling conditions, and the limit of detection of the analytical methodology employed are of paramount importance for accurate estimations. In addition, other limitations such as co-extraction of interfering substances and incomplete

458 extraction have been reported when determining BHT in foods (Karovicová and Simko
459 2000).

460 On the other hand, analytical determination of current levels of BHT in foods could
461 avoid an over-estimation due to the above-mentioned possible losses of BHT during food
462 processing at high temperatures, such as heating or frying (Warner and others 1986a;
463 Leclercq and others 2000). In fact, most of the BHT-containing foods that are taken into
464 account in dietary intake studies are heat-treated products (Table 2). It must also be noted
465 that over-estimation derived from the consideration of MPLs of BHT in authorized food
466 products could be counterbalanced by under-estimation given that the food categories
467 selected in most of the studies exclude other foodstuffs in which BHT is not allowed,
468 although they might contain this additive as a result of carry-over from ingredients
469 employed for their manufacture (Soubra and others 2007) or by other causes.

470 Moreover, these studies can have additional limitations, since most of them do not
471 estimate either the amount of BHT actually consumed by heavy eaters, also called
472 extreme consumers (Maziero and others 2001), or consumer brand loyalty data (Vin and
473 others 2013).

474 According to Table 2, exposure to BHT in most of the studies is unlikely to exceed the
475 current ADI of 0-0.25 mg/kg bw/day. However, some exceptions can be found.
476 Kirkpatrick and Lauer (1986) reported that Canadian children aged 1-4 were exposed to
477 0.39 mg/kg bw/day, especially due to their consumption of cereals, sugars, and potatoes.
478 Leclercq and others (2000) indicated that the Italian population exposure to BHT was up
479 to 0.315 mg/kg bw/day, the main potential sources of BHT being "pastry, cake, and
480 biscuits", "chewing gums", and "vegetable oils and margarine"; overall they contributed
481 74% of the exposure. The JECFA (2000) estimated that the population of the USA was

exposed to 0.39 and 0.78 mg/kg bw/day for mean and high levels of consumption respectively. Although it is not shown in Table 2, the EFSA (2012) Panel noted that the exposure of children aged 3-9 to BHT in Finland and The Netherlands exceeded the ADI (0.040-0.296 mg/kg bw/day) at the 95 th percentile level (high levels of exposure). More recently, Vin and others (2013) estimated the dietary exposure to various additives in France, Italy, UK, and Ireland and reported that mean intakes of BHT exceeded the ADI only for Irish children. In addition to the above-mentioned different methodological approaches and to the selection of foodstuffs subject of study, the comparison of different countries shows that exposure greatly depends on the age group, the eating habits, and the manufacturing practices. As consumer behavior and manufacturers´ additive preferences might change in time, and considering also the widespread use of BHT, constant monitoring of its intake by food safety authorities is needed.

Finally, it must be noted that despite the toxicological aspects discussed above, to the best of our knowledge no estimation of the dietary intake of BHT metabolites has been carried out yet. In relation to this, the case of the additive ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) must be mentioned, because its ADI is calculated considering the dietary intake of itself and of three of its metabolites/degradation products (JMPR 2005). This compound is another synthetic antioxidant widely used in animal feed. It cannot be used in any food for human consumption, but like BHT it can pass from feed to farmed fish, poultry, and eggs, so humans can be exposed to it and to its metabolites.

8. Additional sources of exposure and regulations

When the dietary intake of BHT is calculated, usually only its presence in foods in which it is regulated by legislation is considered. Nevertheless, due to the variety of uses of BHT and its ubiquitous presence, there are other additional sources of exposure that should also be taken into account, such as the carry-over from animal feed to food, its presence in smoke flavorings, its migration from plastic pipelines and packaging to drinking water and other foodstuffs, or its presence in the natural environments from which it can reach the food chain.

Due to the use of BHT also as a feed additive for all animal species (except dogs) at a maximum concentration of 150 mg/kg, either alone or in combination with BHA and/or ethoxyquin (EU Regulation (EC) No. 1831/2003), its potential carry-over from feed to farmed animals requires special attention from a food safety point of view. Thus, residual amounts of the parent compound and/or its metabolites may be ingested by humans from a wide variety of animal products. Although the maximum residue limits (MRLs) of BHT have been established as 10 mg/kg for fish, 3 mg/kg for chicken fat, and 0.5 mg/kg for pig fat in some countries like Japan (JMHLW 2005), in the European Union these MRLs do not currently exist for synthetic antioxidants in food products of animal origin.

As far back as 1965 it was reported that after feeding chickens for 10 weeks with a diet containing 200 mg/kg of BHT their edible portions contained 1-3 mg/kg of BHT and its metabolites (unspecified), and even higher concentrations in isolated fat, skin, digestive organs, and viscera. The eggs from hens fed similar diets contained approximately 2 mg/kg (Frawley and others 1965). In addition, regarding processed meat products, the presence of BHT and its metabolites BHT-OH and BHT-QM in a Spanish dry fermented sausage was demonstrated (Ansorena and others 2001). Their presence was attributed to the addition of BHT either to the pig feed or to the sausage mixture. More recently, due

529 to the growth of aquaculture, several authors have focused on the transfer of synthetic
530 antioxidants and their metabolites from feed to farmed fish. Levels of these compounds
531 in fish appeared to be fairly dose-dependent during farming and mainly present in high-
532 fat-content tissues, such as liver, but also in muscle tissue. Holaas and others (2008)
533 studied the liver deposition and toxicological effects in Atlantic salmon fed graded levels
534 of BHT during a 12-week feeding followed by a 2-week depuration period. As a safety
535 precaution, the production of farmed Atlantic salmon in Norway requires a mandatory 2-
536 week depuration period prior to slaughtering and market delivery, in order to ensure the
537 elimination of veterinary medications, additives, and other undesirable components. It
538 was demonstrated that BHT was highly retained in fish liver and that only 8-13% of fed
539 BHT was eliminated during the depuration period. The authors also suggested that, during
540 starvation, lipids stored in adipose tissue are utilized and may provoke the release of BHT
541 into the circulatory system, thus affecting hepatic metabolism functions. The importance
542 of these results was highlighted, since these residual levels of BHT can pose a
543 toxicological threat for fish consumers. Lundebye and others (2010) analyzed the levels
544 of BHT, BHA, and ethoxyquin in feed and in farmed fish, namely Atlantic salmon,
545 Atlantic halibut (*Hippoglossus hippoglossus*), cod (*Gadus morhua*), and rainbow trout
546 (*Oncorhynchus mykiss*). The highest levels of BHT (7.6 mg/kg) were found in salmon
547 fillets, slightly lower concentrations were found in those of halibut and rainbow trout, and
548 levels were below the detection limits in cod fillets, which is a low-fat fish. The theoretical
549 consumer exposure to the synthetic antioxidants from the consumption of farmed fish was
550 calculated, and it was calculated that a 300-g portion of farmed Atlantic salmon would
551 contribute up to 75% of the ADI for BHT (in that year the ADI had been set at 0.30 mg/kg
552 bw/day). More recently, the Norwegian National Institute of Nutrition and Seafood
553 Research analyzed the content of BHT and other additives, nutrients, and undesirable

554 compounds in commercial fish feeds in the years 2000-2010, in order to detect potential
555 changes over time (Sissener and others 2013). Levels of BHT in feed ranging from
556 approximately 5 to 20 mg/kg were detected; the sum, together with BHA and ethoxyquin,
557 was always below the legal limit. Nevertheless, the BHT concentrations obtained in 2000-
558 2010 were highly variable and very little of the total variation was explicable by trends
559 over time. Guillén and Errecalde (2002) also reported the presence of BHT in farmed
560 rainbow trout.

561 Furthermore, foodstuffs smoked with liquid smoke flavorings might supposedly be
562 another additional source of exposure to BHT. This compound and its metabolites BHT-
563 Q and BHT-CHO were detected in a commercial liquid smoke flavoring (Guillén and
564 Ibargoitia 1998, 1999). The presence of BHT was attributed to its pyrolytic formation in
565 the smoke generation process. In this context, BHT and its metabolite BHT-CHO were
566 also detected in oak wood (Guillén and Manzanos 2001), a common hardwood rich in
567 lignin used to manufacture smoke flavorings. Recently a study on the generation of BHT
568 from lignin degradation was carried out with the purpose of converting lignin into
569 valuable products (Zhang and others 2014). Moreover, BHT has been identified in some
570 ready-to-eat fish products, either of farmed or wild origin, which had been smoked with
571 natural smoke (Guillén and Errecalde 2002; Guillén and others 2006).

572 Another additional source of exposure to BHT and its metabolites is derived from its
573 use as an additive in plastic materials employed for water pipelines or food-packaging.
574 Due to the limited stability of polyolefins at high temperatures and ultraviolet light,
575 antioxidants are commonly added (Al-Malaika 2004), but, unfortunately, the migration
576 of these compounds and their degradation products from the polymer matrix into the food
577 or water content can occur (Dopico-García and others 2007). Thus, when commercially

bottled drinking water was studied, the presence of BHT was detected in 46% of samples (Tombesi and Freije 2002). In a newly installed pipeline distribution system the presence of BHT-Q and BHT-CHO was reported and the latter metabolite was identified in the drinking water samples collected from it (Brocca and others 2002). In agreement with these results, other authors detected BHT, DBP, and BHT-Q in drinking water collected from plastic pipes (Skjevrak and others 2003; Lützhøft and others 2013). It must be noted that in the last re-evaluation of BHT as a food additive (EFSA 2012), the Panel concluded that this use of BHT in food contact materials would contribute an additional human exposure of 0.05 mg/kg bw/day. This value was obtained assuming that every day throughout his/her lifetime a person weighing 60 kg consumes 1 kg of food packed in plastics containing BHT at its maximum permitted level (specific migration limit of 3 mg/kg food). It is worth noting that exposure of children at mean and at the 95th percentile to BHT would exceed the current ADI if this additional source was taken into account (EFSA 2012).

Finally, the environmental occurrence of BHT and its metabolites in rivers, rain, ground water, and waste waters can also be an additional source of exposure, because these compounds can easily reach the food chain by these means. Fries and Püttmann (2002, 2004) detected BHT and BHT-CHO in river, ground, rain, roof-runoff, and waste waters. In aquatic environments two major sources of these compounds were suggested, both related to industries that manufacture solid products containing antioxidants, such as food, cosmetics, plastics, and rubber: first and foremost, through waste water discharge and, secondly, by means of the potential evaporation of BHT into the atmosphere due to its low solubility in water and its high volatility. The dimer 2-BHT was only identified in ground water samples, probably generated through anaerobic biodegradation of BHT by microorganisms in ground water or soil. BHT and its metabolites BHT-Q, BHT-CH_2OH,

603 BHT-CHO, and BHT-COOH were detected in raw waste water samples, BHT-COOH
604 being found at higher concentrations than the others (Rodil and others 2010, 2012).

605

606 ## 9. Analytical determination in foods

607 The analytical methods employed to evaluate the presence of BHT in foodstuffs have
608 recently been reviewed in detail by several authors (García and others 2006; André and
609 others 2010; Serra and others 2013). Nevertheless, the same cannot be said regarding its
610 metabolites. In this context, **Table 3** summarizes the analytical techniques used to
611 determine their occurrence in different foods and related matrixes. It can be observed that
612 not only are the techniques and the ranges of abundance of the metabolites very varied,
613 but so are the matrixes under study, with water being the most frequently examined.
614 Therefore, the establishment of a method for the simultaneous determination of all
615 possible BHT metabolites present in foodstuffs would constitute a great advance in
616 evaluating not only the occurrence of all these potentially toxic compounds in food, but
617 also their incidence in the human diet.

618

619 ## 10. Conclusions

620 The ubiquitous presence of BHT, its controversial toxicological data, a lack of
621 information about its true dietary intake, and also that of its metabolites have increased
622 consumer concern about the use of this synthetic food additive. Further research is needed
623 to evaluate the current extent of human exposure to BHT and its metabolites, not only as
624 a result of their presence in authorized foods, but also as related to other additional sources
625 that reach the food chain, such as carry-over processes from feed to farmed animal

626 products, migration from plastic pipelines and packaging to water and food, and their
627 presence in smoke flavorings and in natural environments. For this purpose, the
628 development of proper analytical techniques to simultaneously determine BHT and all
629 possible metabolites is of paramount importance. Moreover, considering the possible dual
630 role of BHT as either antioxidant or prooxidant, further research is needed on this topic,
631 paying special attention to the conditions and mechanisms by which BHT acts on foods
632 in these two ways.

633

634 **11. Acknowledgments**

635 This work was supported by the Spanish Ministry of Economy and Competitiveness
636 (MINECO, AGL2012-36466), the Basque Government (EJ-GV, GIC10/85-IT-463-10),
637 and the UPV/EHU (UFI-11/21). Author B. N-E. thanks the UPV/EHU for a predoctoral
638 fellowship.

639

12. References

Al-Akid YF, El-Rahman AEA, Hussein HA, Wassif GA. 2001. Nephro- and pneumotoxic response to chronic administration of butylated hydroxytoluene (BHT) in adult albino rats. Al-Azhar J Pharm Sci 28:171-95.

Al-Malaika S. 2004. Perspectives in stabilisation of polyolefins. In: Albertsson AC, editor. Long-term properties of polyolefins. Berlin: Springer. p 121-50.

Allam SSM, Mohamed HMA. 2002. Thermal stability of some commercial natural and synthetic antioxidants and their mixtures. J Food Lipids 9(4):277-93. DOI: 10.1111/j.1745-4522.2002.tb00226.x

Allen JR. 1976. Long-term antioxidant exposure effects on female primates. Arch Environ Health 31(1):47-50. DOI: 10.1080/00039896.1976.10667189

André C, Castanheira I, Cruz JM, Paseiro P, Sanches-Silva A. 2010. Analytical strategies to evaluate antioxidants in food: a review. Trends Food Sci Tech 21(5):229-46. DOI: 10.1016/j.tifs.2009.12.003

Ansorena D, Gimeno O, Astiasarán I, Bello J. 2001. Analysis of volatile compounds by GC-MS of a dry fermented sausage: chorizo de Pamplona. Food Res Int 34(1):67-75. DOI: 10.1016/S0963-9969(00)00133-2

Aruoma OI. 1991. Pro-oxidant properties: an important consideration for food additives and/or nutrient components? In: Aruoma OI, Halliwell B, editors. Free radicals and food additives. London: Taylor and Francis Ltd. p 173-94.

Augustin MA, Berry SK. 1983. Efficacy of the antioxidants BHA and BHT in palm olein during heating and frying. J Am Oil Chem Soc 60(8):1520-3. DOI: 10.1007/BF02666575

Awah FM, Eyibe UN, Ikhelowa AO, Anyimigbo GC, Nkuche BC. 2012. Protective capabilities of rutin in acute butylated hydroxytoluene-induced oxidative damage in Wistar rats. Int J Pharm Front 2(2):12-20.

Babich H. 1982. Butylated hydroxytoluene (BHT): A review. Environ Res 29(1):1-29. DOI: 10.1016/0013-9351(82)90002-0

Babu B, Wu JT. 2008. Production of natural butylated hydroxytoluene as an antioxidant by fresh water phytoplankton. J Phycol 44:1447-54. DOI: 10.1111/j.1529-8817.2008.00596.x

Becker HD. 1969. Quinone dehydrogenation. IV. One-electron oxidations with 2,3-dichloro-5,6-dicyanobenzoquinone. J Org Chem 34(5):1203-10. DOI: 10.1021/jo01257a005

Bemrah N, Leblanc JC, Volatier JL. 2008. Assessment of dietary exposure in the French population to 13 selected food colours, preservatives, antioxidants, stabilizers, emulsifiers and sweeteners. Food Addit Contam Part B Surveill 1(1):2-14. DOI: 10.1080/19393210802236943

Bianchi L, Colivicchi MA, Della Corte L, Valoti M, Sgaragli GP, Bechi P. 1997. Measurement of synthetic phenolic antioxidants in human tissues by high-performance liquid chromatography with coulometric electrochemical detection. J Chromatogr B 694(2):359-65. DOI: 10.1016/S0378-4347(97)00150-3

682 Björkhem I, Henriksson-Freyschuss A, Breuer O, Diczfalusy U, Berglund L, Henriksson
683 P. 1991. The antioxidant butylated hydroxytoluene protects against atherosclerosis.
684 Arterioscler Thromb Vasc Biol 11:15-22. DOI: 10.1161/01.ATV.11.1.15
685 Bolton JL, Sevestre H, Ibe BO, Thompson JA. 1990. Formation and reactivity of
686 alternative quinone methides from butylated hydroxytoluene: possible explanation for
687 species-specific pneumotoxicity. Chem Res Toxicol 3(1):65-70. DOI:
688 10.1021/tx00013a011
689 Bomhard EM, Bremmer JN, Herbold BA. 1992. Review of the mutagenicity/genotoxicity
690 of butylated hydroxytoluene. Mutat Res 277(3):187-200. DOI: 10.1016/0165-
691 1110(92)90043-9
692 Botterweck AA, Verhagen H, Goldbohm RA, Kleinjans J, van den Brandt PA. 2000.
693 Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer
694 risk: results from analyses in the Netherlands Cohort Study. Food Chem Toxicol
695 38(7):599-605.
696 Branen AL, Richardson T, Goel MC, Allen JR. 1973. Lipid and enzyme changes in the
697 blood and liver of monkeys given butylated hydroxytoluene and butylated
698 hydroxyanisole. Food Cosmet Toxicol 11(5):797-806. DOI: 10.1016/S0015-
699 6264(73)80363-3
700 Branen AL. 1975. Toxicology and biochemistry of butylated hydroxyanisole and
701 butylated hydroxytoluene. J Am Oil Chem Soc 52(2):59-63. DOI:
702 10.1007/BF02901825
703 Brocca D, Arvin E, Mosbaek H. 2002. Identification of organic compounds migrating
704 from polyethylene pipelines into drinking water. Water Res 36(15):3675-80. DOI:
705 10.1016/S0043-1354(02)00084-2
706 Brugh MJr. 1977. Butylated hydroxytoluene protects chickens exposed to Newcastle
707 disease virus. Science 197(4310):1291-2. DOI:10.1126/science.897670
708 Chevillard G, Nouhi Z, Anna D, Paquet M, Blank V. 2010. Nrf3-deficient mice are not
709 protected against acute lung and adipose tissue damages induced by butylated
710 hydroxytoluene. FEBS Lett 584(5):923-8. DOI: 10.1016/j.febslet.2010.01.028.
711 Clapp NK, Tyndall RL, Cumming RB. 1973. Hyperplasia of hepatic bile ducts in mice
712 following long-term administration of butylated hydroxytoluene. Food Cosmet
713 Toxicol 11(5):847-9. DOI: 10.1016/0015-6264(73)90143-0
714 Collings AJ, Sharratt M. 1970. The BHT content of human adipose tissue. Food Cosmet
715 Toxicol 8(4):409-12. DOI: 10.1016/S0015-6264(70)80393-5
716 Commission of the EC-Commission of the European Communities. 1989. Reports of the
717 Scientific Committee for Food, Twenty-second Series. Available from:
718 http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_22.pdf. Accessed 2014 July 02.
719 Conacher HB, Iverson F, Lau PY, Page BD. 1986. Levels of BHA and BHT in human
720 and animal adipose tissue: interspecies extrapolation. Food Chem Toxicol 24(10-
721 11):1159-62. DOI: 10.1016/0278-6915(86)90302-9
722 Conning DM, Phillips JC. 1986. Comparative metabolism of BHA, BHT and other
723 phenolic antioxidants and its toxicological relevance. Food Chem Toxicol 24(10-
724 11):1145-8. DOI: 10.1016/0278-6915(86)90300-5

725 Dacre JC. 1961. The metabolism of 3:5-di-*tert*-butyl-4-hydroxytoluene and 3:5-di-*tert*-
726 butyl-4-hydroxybenzoic acid in the rabbit. Biochem J 78(4):758-66.
727 Daniel JW, Gage JC. 1965. The absorption and excretion of butylated hydroxytoluene
728 (BHT) in the rat. Food Cosmet Toxicol 3:405-15. DOI: 10.1016/S0015-
729 6264(65)80128-6
730 Daniel JW, Gage JC, Jones DI, Stevens MA. 1967. Excretion of butylated hydroxytoluene
731 (BHT) and butylated hydroxyanisole (BHA) by man. Food Cosmet Toxicol 5:475-9.
732 DOI: 10.1016/S0015-6264(67)83148-1
733 Daniel JW, Gage JC, Jones DI. 1968. The metabolism of 3,5-di-*tert*-butyl-4-
734 hydroxytoluene in the rat and in man. Biochem J 106(4):783-90.
735 Daniel JW. 1986. Metabolic aspects of antioxidants and preservatives. Xenobiotica
736 16(10-11):1073-8. DOI: 10.3109/00498258609038984
737 Dopico-García MS, López-Vilariñó JM, Gonzalez-Rodríguez MV. 2007. Antioxidant
738 content of and migration from commercial polyethylene, polypropylene, and polyvinyl
739 chloride packages. J Agric Food Chem 55(8):3225-31. DOI: 10.1021/jf070102
740 European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food
741 additives other than colours and sweeteners.
742 European Parliament and Council Regulation (EC) No. 1831/2003 on additives for use in
743 animal nutrition.
744 European Parliament and Council Regulation (EC) No 1333/2008 of 16 December 2008
745 on food additives
746 EFSA-European Food Safety Authority. Panel on Food Additives and Nutrient Sources
747 added to Food (ANS). 2012. Scientific opinion on the re-evaluation of butylated
748 hydroxytoluene BHT (E 321) as a food additive. EFSA Journal 10(3):2588:1-43. DOI:
749 10.2903/j.efsa.2012.2588.
750 Faine LA, Rodrigues HG, Galhardi CM, Ebaid GMX, Diniz YS, Fernandes AAH, Novelli
751 ELB. 2006. Butyl hydroxytoluene (BHT)-induced oxidative stress: effects on serum
752 lipids and cardiac energy metabolism in rats. Exp Toxicol Pathol 57(3):221-6. DOI:
753 10.1016/j.etp.2005.10.001
754 Fernandez-Alvarez M, Lores M, Jover E, Garcia-Jares C, Bayona JM, Llompart M. 2009.
755 Photo-solid-phase microextraction of selected indoor air pollutants from office
756 buildings. Identification of their photolysis intermediates. J Chromatogr A
757 1216(51):8969-78. DOI: 10.1016/j.chroma.2009.10.047
758 Frankel EN. 2007. Antioxidants in food and biology: facts and fiction. Bridgwater, UK:
759 The Oily Press.
760 Frawley JP, Kay JH, Calandra JC. 1965. The residue of butylated hydroxytoluene (BHT)
761 and metabolites in tissue and eggs of chickens fed diets containing radioactive BHT.
762 Food Cosmet Toxicol 3(3):471-4. DOI: 10.1016/S0015-6264(65)80133-X
763 Fries E, Püttmann W. 2002. Analysis of the antioxidant butylated hydroxytoluene (BHT)
764 in water by means of solid-phase extraction combined with GC/MS. Water Res
765 36(9):2319-27. DOI: 10.1016/S0043-1354(01)00453-5
766 Fries E, Püttmann W. 2004. Monitoring of the antioxidant BHT and its metabolite BHT-
767 CHO in German river water and ground water. Sci Total Environ 319(1-3):269-82.
768 DOI: 10.1016/S0048-9697(03)00447-9

Fujisawa S, Kadoma Y, Yokoe I. 2004. Radical-scavenging activity of butylated hydroxytoluene (BHT) and its metabolites. Chem Phys Lipids 130(2):189-95. DOI: 10.1016/j.chemphyslip.2004.03.005

Gage JC. 1966. The metabolism of phenolic antioxidants. Fett Wiss Technol 68(11):951-4. DOI: 10.1002/lipi.19660681105

García RS, Silva AS, Cooper I, Franz R, Losada PP. 2006. Revision of analytical strategies to evaluate different migrants from food packaging materials. Trends Food Sci Tech 17(7):354-66. DOI: 10.1016/j.tifs.2006.01.005

Geyer H, Scheunert I, Korte F. 1986. Bioconcentration potential of organic environmental chemicals in humans. Regul Toxicol Pharmacol 6(4):313-47.

Goicoechea E, Van Twillert K, Duits M, Brandon EDFA, Kootstra PR, Blokland MH, Guillén MD. 2008. Use of an *in vitro* digestion model to study the bioaccessibility of 4-hydroxy-2-nonenal and related aldehydes present in oxidized oils rich in omega-6 acyl groups. J Agric Food Chem 56(18):8475-83. DOI: 10.1021/jf801212k

Grogan MW. 1986. Toxicity from BHT ingestion. Western J Med 145(2):245-6.

Guillén MD, Ibargoitia ML. 1998. New components with potential antioxidant and organoleptic properties, detected for the first time in liquid smoke flavoring preparations. J Agric Food Chem 46(4):1276-85. DOI: 10.1021/jf970952x

Guillén MD, Ibargoitia ML. 1999. GC/MS analysis of lignin monomers, dimers and trimers in liquid smoke flavourings. J Sci Food Agric 79:1889-903. DOI: 10.1002/(SICI)1097-0010(199910)79:13<1889::AID-JSFA451>3.0.CO;2-3

Guillén MD, Manzanos MJ. 2001. Some compounds detected for the first time in oak wood extracts by GC/MS. Sci Aliment 21:65-70. DOI: 10.3166/sda.21.65-70

Guillén MD, Errecalde MC. 2002. Volatile components of raw and smoked black bream (*Brama raii*) and rainbow trout (*Oncorhynchus mykiss*) studied by means of solid-phase microextraction and gas chromatography/mass spectrometry. J Sci Food Agric 82:945-52. DOI: 10.1002/jsfa.1128

Guillén MD, Errecalde MC, Salmerón J, Casas C. 2006. Headspace volatile components of smoked swordfish (*Xiphias gladius*) and cod (*Gadus morhua*) detected by means of solid-phase microextraction and gass chromatography-mass spectrometry. Food Chem 94:151-6. DOI: 10.1016/j.foodchem.2005.01.014

Guyton KZ, Bhan P, Kuppusamy P, Zweier JL, Trush MA, Kensler TW. 1991. Free radical-derived quinone methide mediates skin tumor promotion by butylated hydroxytoluene hydroperoxide: expanded role for electrophiles in multistage carcinogenesis. Proc Natl Acad Sci USA 88(3):946-50. DOI: 10.1073/pnas.88.3.946

Halliwell B. 2011. Free radicals and antioxidants - quo vadis? Trends Pharmacol Sci 32(3):125-30.

Hamama AA, Nawar WW. 1991. Thermal decomposition of some phenolic antioxidants. J Agric Food Chem 39(6):1063-9. DOI: 10.1021/jf00006a012

Hernández F, Portolés T, Pitarch E, López FJ. 2009. Searching for anthropogenic contaminants in human breast adipose tissues using gas chromatography-time-of-flight mass spectrometry. J Mass Spectrom 44(1):1-11. DOI: 10.1002/jms.1538

Hirose M, Shibata M, Hagiwara A, Imaida K, Ito N. 1981. Chronic toxicity of butylated hydroxytoluene in Wistar rats. Food Cosmet Toxicol 19:147-51. DOI: 10.1016/0015-6264(81)90350-3

Holaas E, Bohne VB, Hamre K, Arukwe A. 2008. Hepatic retention and toxicological responses during feeding and depuration periods in Atlantic salmon (*Salmo salar*) fed graded levels of the synthetic antioxidant, butylated hydroxytoluene. J Agric Food Chem 56(23):11540-9. DOI: 10.1021/jf8025524

Holder GM, Ryan AJ, Watson TR, Wiebe LI. 1970a. The biliary metabolism of butylated hydroxytoluene (3,5-di-*t*-butyl-4-hydroxy-toluene) and its derivatives in the rat. J Pharm Pharmacol 22(11):832-8. DOI: 10.1111/j.2042-7158.1970.tb08448.x

Holder GM, Ryan AJ, Watson TR., Wiebe LI. 1970b. The metabolism of butylated hydroxytoluene, (3,5-di-*t*-butyl-4-hydroxytoluene) in man. J Pharm Pharmacol 22(5):375-6. DOI: 10.1111/j.2042-7158.1970.tb08541.x

IARC-International Agency for Research on Cancer. 1987. Some naturally occurring and synthetic food components, furocoumarins and ultraviolet radiation summary of data reported and evaluation. IARC Monographs on the evaluation of carcinogenic risks to humans 40. Available from: http://monographs.iarc.fr/ENG/Monographs/vol1-42/mono40.pdf. Accessed 2014 July 02.

Inai K, Kobuke T, Nambu S, Takemoto T, Kou E, Nishina H, Fujihara M, Yonehara S, Suehiro S, Tsuya T. 1988. Hepatocellular tumorigenicity of butylated hydroxytoluene administered orally to B6C3F1 mice. Jpn J Cancer Res 79(1):49-58.

JECFA-Joint FAO/WHO Expert Committee on Food Additives. 1996. Butylated hydroxytoluene. Toxicologycal evaluation of certain food additives and contaminants in food. WHO Food additives Series 35. Available from: http://www.inchem.org/documents/jecfa/jecmono/v35je02.htm. Accessed 2014 July 02.

JECFA-Joint FAO/WHO Expert Committee on Food Additives. 2000. Evaluation of certain food additives (Fifty-first report). WHO-World Health Organization Technical Report Series, No. 891. Available from: http://whqlibdoc.who.int/trs/WHO_TRS_891.pdf?ua=1. Accessed 2014 July 02.

Jiang G, Lin S, Wen L, Jiang Y, Zhao M, Chen F, Prasad KN, Duan X, Yang B. 2013. Identification of a novel phenolic compound in litchi (*Litchi chinensis* Sonn.) pericarp and bioactivity evaluation. Food Chem 136:563-8. DOI: 10.1016/j.foodchem.2012.08.089

JMHLW-Japanese Ministry of Health, Labour and Welfare. 2005. The Japan food chemical research foundation: maximum residue limits (MRLs) list: compositional specification for foods. Available from: http://www.m5.ws001.squarestart.ne.jp/foundation/agrdtl.php?a_inq=30500. Accessed 2014 July 02.

JMPR-Joint FAO/WHO. 2005. Pesticide residues in food. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Available from: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/JMPR05report.pdf. Accessed 2014 July 02.

855 Kagan VE, Serbinova EA, Packer L. 1990. Generation and recycling of radicals from
856 phenolic antioxidants. Arch Biochem Biophys 280(1):33-9.
857 Kahl R. 1984. Synthetic antioxidants: biochemical actions and interference with
858 radiation, toxic compounds, chemical mutagens and chemical carcinogens.
859 Toxicology 33(3-4):185-228. DOI: 10.1016/0300-483X(84)90038-6
860 Karovicová J, Simko P. 2000. Determination of synthetic phenolic antioxidants in food
861 by high-performance liquid chromatography. J Cromatogr A 882(1-2):271-81.
862 Kharasch MS, Joshi BS. 1957. Reactions of hindered phenols. II. Base-catalyzed
863 oxidations of hindered phenols. J Org Chem 22(11):1439-43. DOI:
864 10.1021/jo01362a034
865 Kirkpatrick DC, Lauer BH. 1986. Intake of phenolic antioxidants from foods in Canada.
866 Food Chem Toxicol 24(10-11):1035-7. DOI: 10.1016/0278-6915(86)90285-1
867 Klein PJ, Van Vleet TR, Hall JO, Coulombe RA. 2003. Effects of dietary butylated
868 hydroxytoluene on aflatoxin B1-relevant metabolic enzymes in turkeys. Food Chem
869 Toxicol 41(5):671-8. DOI: 10.1016/S0278-6915(02)00332-0
870 Kupfer R, Dwyer-Nield LD, Malkinson AM, Thompson JA. 2002. Lung toxicity and
871 tumor promotion by hydroxylated derivatives of 2,6-di-*tert*-butyl-4-methylphenol
872 (BHT) and 2-*tert*-butyl-4-methyl-6-iso-propylphenol: correlation with quinone
873 methide reactivity. Chem Res Toxicol 15(8):1106-12. DOI: 10.1021/tx0255525
874 Ladomery LG, Ryan AJ, Wright SE. 1967. The biliary metabolites of butylated
875 hydroxytoluene in the rat. J Pharm Pharmacol 19(6):388-94. DOI: 10.1111/j.2042-
876 7158.1967.tb09565.x
877 Lambert CR, Black HS, Truscott, TG. 1996. Reactivity of butylated hydroxytoluene. Free
878 Radic Biol Med 21(3):395-400.
879 Leclercq C, Arcella D, Turrini A. 2000. Estimates of the theoretical maximum daily
880 intake of erythorbic acid, gallates, butylated hydroxyanisole (BHA) and butylated
881 hydroxytoluene (BHT) in Italy: a stepwise approach. Food Chem Toxicol
882 38(12):1075-84. DOI: 10.1016/S0278-6915(00)00106-X
883 Lemercier JN, Meier BW, Gomez JD, Thompson JA. 2004. Inhibition of glutathione S-
884 transferase P1-1 in mouse lung epithelial cells by the tumor promoter 2,6-di-*tert*-butyl-
885 4-methylene-2,5-cyclohexadienone (BHT-quinone methide): protein adducts
886 investigated by electrospray mass spectrometry. Chem Res Toxicol 17(12):1675-83.
887 DOI: 10.1021/tx049811x
888 Leventhal B, Daun H, Gilbert SG. 1976. Isolation and identification of 3,3′,5,5′-tetra-bis-
889 (*tert*-butyl)-stilbenequinone from edible oil with added BHT. J Food Sci 41(2):467-8.
890 DOI: 10.1111/j.1365-2621.1976.tb00648.x
891 Lin HM, Yen FL, Ng LT, Lin CC. 2007. Protective effects of *Ligustrum lucidum* fruit
892 extract on acute butylated hydroxytoluene-induced oxidative stress in rats. J
893 Ethnopharmacol 111(1):129-36. DOI: 10.1016/j.jep.2006.11.004
894 Lindenschmidt RC, Tryka AF, Goad ME, Witschi HP. 1986. The effects of dietary
895 butylated hydroxytoluene on liver and colon tumor development in mice. Toxicology
896 38(2):151-60. DOI: 10.1016/0300-483X(86)90116-2
897 Lundebye A-K, Hove H, Måge A, Bohne VJB, Hamre K. 2010. Levels of synthetic
898 antioxidants (ethoxyquin, butylated hydroxytoluene and butylated hydroxyanisole) in

fish feed and commercially farmed fish. Food Addit Contam 27(12):1652-7. DOI: 10.1080/19440049.2010.508195
Lützhøft HCH, Waul CK, Andersen HR, Seredynska-Sobecka B, Mosbæk H, Christensen N, Olsson ME, Arvin E. 2013. HS-SPME-GC-MS analysis of antioxidant degradation products migrating to drinking water from PE materials and PEX pipes. Int J Environ An Ch 93(6):593-612. DOI: 10.1080/03067319.2012.727805
Malkinson AM. 1983. Review: putative mutagens and carcinogens in foods. III. Butylated hydroxytoluene (BHT). Environ Mutagen 5(3):353-62. DOI: 10.1002/em.2860050313
Malkinson AM, Koski KM, Evans WA, Festing MFW. 1997. Butylated hydroxytoluene exposure is necessary to induce lung tumors in BALB mice treated with 3-methylcholanthrene. Cancer Res 57(14):2832-4.
Marmesat S, Morales A, Velasco J, Dobarganes MC. 2010. Action and fate of natural and synthetic antioxidants during frying. Grasas Aceites 61(4):333-40. DOI: 10.3989/gya.021910
Matsuo M, Mihara K, Okuno M, Ohkawa H, Miyamoto J. 1984. Comparative metabolism of 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) in mice and rats. Food Chem Toxicol 22(5):345-54. DOI: 10.1016/0278-6915(84)90362-4
Maziero GC, Baunwart C, Toledo MC. 2001. Estimates of the theoretical maximum daily intake of phenolic antioxidants BHA, BHT and TBHQ in Brazil. Food Addit Contam 18(5):365-73. DOI: 10.1080/02652030120645
Meier BW, Gomez JD, Kirichenko OV, Thompson JA. 2007. Mechanistic basis for inflammation and tumor promotion in lungs of 2,6-di-*tert*-butyl-4-methylphenol-treated mice: electrophilic metabolites alkylate and inactivate antioxidant enzymes. Chem Res Toxicol 20(2):199-207. DOI: 10.1021/tx060214f
Mizutani T, Nomura H, Nakanishi K, Fujita S. 1987. Hepatotoxicity of butylated hydroxytoluene and its analogs in mice depleted of hepatic glutathione. Toxicol Appl Pharm 87(1):166-76. DOI: 10.1016/0041-008X(87)90094-9
Nagai F, Ushiyama K, Kano I. 1993. DNA cleavage by metabolites of butylated hydroxytoluene. Arch Toxicol 67(8):552-7. DOI: 10.1007/BF01969268
Nanou K, Roukas T. 2010. Oxidative stress response and morphological changes of *Blakeslea trispora* induced by butylated hydroxytoluene during carotene production. Appl Biochem Biotechnol 160(8):2415-23. DOI: 10.1007/s12010-009-8712-y
Oikawa S, Nishino K, Oikawa S, Inoue S, Mizutani T, Kawanishi S. 1998. Oxidative DNA damage and apoptosis induced by metabolites of butylated hydroxytoluene. Biochem Pharmacol 56(3):361-70. DOI: 10.1016/S0006-2952(98)00037-9
Olsen P, Meyer O, Bille N, Würtzen G. 1986. Carcinogenicity study on butylated hydroxytoluene (BHT) in Wistar rats exposed in utero. Food Chem Toxicol 24(1):1-12. DOI: 10.1016/0278-6915(86)90256-5
Philip M, Rowley DA, Schreiber H. 2004. Inflammation as a tumor promoter in cancer induction. Semin Cancer Biol 14(6):433-9. DOI: 10.1016/j.semcancer.2004.06.006
Price SC. 1994. The role of hepatocellular injury in the chronic toxicity of BHT: Two-generation Wistar albino rat study. Robens Institute, U. of Surrey, Guilford, Surrey,

U. K. Study No: 1/91/Tx. Final Report No: R193/TOX/0020. Vol. 1-8. Submitted to WHO by Robens Institute. Unpublished.

Rao GVS, Parthasarathy KR, Sundararaj A. 2000. Haemorrhagic syndrome in butylated hydroxy toluene (BHT) toxicity in broiler chicken. Indian Vet J 77(2):117-19.

Rodil R, Quintana JB, Basaglia G, Pietrogrande MC, Cela R. 2010. Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography-mass spectrometry. J Chromatogr A 1217(41):6428-35. DOI: 10.1016/j.chroma.2010.08.020

Rodil R, Quintana JB, Cela R. 2012. Oxidation of synthetic phenolic antioxidants during water chlorination. J Hazard Mater 199-200:73-81. DOI: 10.1016/j.jhazmat.2011.10.058

Santos NA, Cordeiro AMTM, Damasceno SS, Aguiar RT, Rosenhaim R, Carvalho Filho JR, Santos IMG, Maia AS, Souza AG. 2012. Commercial antioxidants and thermal stability evaluations. Fuel 97:638-43. DOI: 10.1016/j.fuel.2012.01.074

Serra A, Macia A, Estevez M. 2013. Synthetic phenolic antioxidants. In: Nollet LML, Toldrá F, editors. Food analysis by HPLC. Boca Raton, Florida: CRC Press. p 551-65.

Shaw YS, Chen C. 1972. Ring hydroxylation of di-*t*-butylhydroxytoluene by rat liver microsomal preparations. Biochem J 128(5):1285-91.

Shearn CT, Fritz KS, Thompson JA. 2011. Protein damage from electrophiles and oxidants in lungs of mice chronically exposed to the tumor promoter butylated hydroxytoluene. Chem Biol Interact 192(3):278-86. DOI: 10.1016/j.cbi.2011.04.005

Shirai T, Hagiwara A, Kurata Y, Shibata M, Fukushima S, Ito N. 1982. Lack of carcinogenicity of butylated hydroxytoluene on long-term administration to B6C3F1 mice. Food Chem Toxicol 20(6):861-5. DOI: 10.1016/S0015-6264(82)80219-8

Shlian DM, Goldstone J. 1986. More on BHT toxicity. Western J Med 145(5):699.

Sissener NH, Julshamn K, Espe M, Lunestad BT, Hemre GI, Waagbo R, Mage A. 2013. Surveillance of selected nutrients, additives and undesirables in commercial Norwegian fish feeds in the years 2000-2010. Aquacult Nutr 19(4):555-72. DOI: 10.1111/anu.12007

Skjevrak I, Due A, Gjerstad KO, Herikstad H. 2003. Volatile organic components migrating from plastic pipes (HDPE, PEX and PVC) into drinking water. Water Res 37(8):1912-20. DOI: 10.1016/S0043-1354(02)00576-6

Slaga TJ. 1995. Inhibition of skin tumor initiation, promotion, and progression by antioxidants and related compounds. Crit Rev Food Sci 35(1-2):51-7. DOI: 10.1080/10408399509527686

Smirnova EG, Lyubimov YI, Malinina TG, Lyubimova EY, Alexandrushkina NI, Vanyushin BF, Kolesova GM, Yaguzhinsky LS. 2002. Ionol (BHT) produces superoxide anion. Biochem Moscow 67(11):1271-5. DOI: 10.1023/A:1021357506703

Snipes W, Person S, Keith A, Cupp J. 1975. Butylated hydroxytoluene inactivated lipid-containing viruses. Science 188(4183):64-6. DOI: 10.1126/science.163494

Soubra L, Sarkis D, Hilan C, Verger Ph. 2007. Dietary exposure of children and teenagers to benzoates, sulphites, butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) in Beirut (Lebanon). Regul Toxicol Pharm 47:68-77. DOI:10.1016/j.yrtph.2006.07.005

Stokes JD, Scudder CL. 1974. The effect of butylated hydroxyanisole and butylated hydroxytoluene on behavioral development of mice. Devel Psychobiol 7(4):343-50.

Suh H J, Chung MS, Cho YH, Kim JW, Kim DH, Han KW, Kim CJ. 2005. Estimated daily intakes of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) and *tert*-butyl hydroquinone (TBHQ) antioxidants in Korea. Food Addit Contam 22(12):1176-88. DOI: 10.1080/02652030500195288

Sun Y, Dwyer-Nield LD, Malkinson AM, Zhang YL, Thompson JA. 2003. Responses of tumorigenic and non-tumorigenic mouse lung epithelial cell lines to electrophilic metabolites of the tumor promoter butylated hydroxytoluene. Chem Biol Interact 145(1):41-51.

Takahashi O. 1992. Haemorrhages due to defective blood coagulation do not occur in mice and guinea pigs fed butylated hydroxytoluene, but nephrotoxicity is found in mice. Food Chem Toxicol 30(2):89-97. DOI: 10.1016/0278-6915(92)90143-9

Tanaka T, Oishi S, Takahashi O. 1993. Three generation toxicity study of butylated hydroxytoluene administered to mice. Toxicol Lett 66(3): 295-304.

Thompson DC, Trush MA. 1986. The toxicological implications of the interaction of butylated hydroxytoluene with other antioxidants and phenolic chemicals. Food Chem Toxicol 24(10-11):1189-95. DOI: 10.1016/0278-6915(86)90307-8

Thompson JA, Carlson TJ, Sun Y, Dwyer-Nield LD, Malkinson AM. 2001. Studies using structural analogs and inbred strain differences to support a role for quinone methide metabolites of butylated hydroxytoluene (BHT) in mouse lung tumor promotion. Toxicology 160(1-3):197-205. DOI: 10.1016/S0300-483X(00)00449-2

Tombesi NB, Freije H. 2002. Application of solid-phase microextraction combined with gas chromatography–mass spectrometry to the determination of butylated hydroxytoluene in bottled drinking water. J Chromatogr A 963(1–2):179-83. DOI: 10.1016/S0021-9673(02)00217-0

Tsaknis J, Lalas S, Protopapa E. 2002. Effectiveness of the antioxidants BHA and BHT in selected vegetable oils during intermittent heating. Grasas Aceites 53(2):199-205. DOI:10.3989/gya.2002.v53.i2.305

Vin K, Connolly A, McCaffrey T, McKevitt A, O'Mahony C, Prieto M, Tennant D, Hearty A, Volatier JL. 2013. Estimation of the dietary intake of 13 priority additives in France, Italy, the UK and Ireland as part of the FACET project. Food Addit Contam A 30(12):2050-80. DOI: 10.1080/19440049.2013.851417

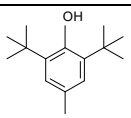
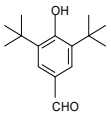
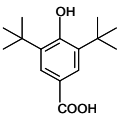
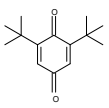
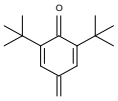
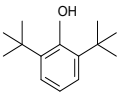
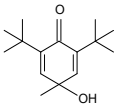
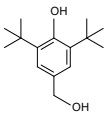
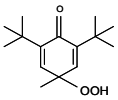
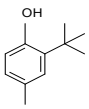
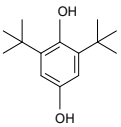
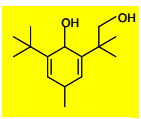
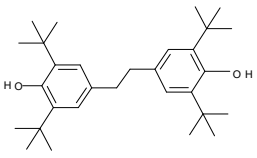
Verhagen H, Beckers HH, Comuth PA, Maas LM, ten Hoor F, Henderson PT, Kleinjans JC. 1989. Disposition of single oral doses of butylated hydroxytoluene in man and rat. Food Chem Toxicol 27(12):765-72.

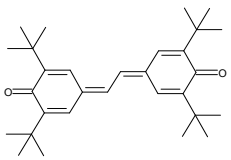
Verhagen H, Deerenberg I, Marx A, Ten Hoor F, Henderson PTh, Kleinjans JCS. 1990. Estimate of the maximal daily dietary intake of butylated hydroxyanisole and butylated hydroxytoluene in The Netherlands. Food Chem Toxicol 28(4):215-20. DOI: 10.1016/0278-6915(90)90033-J

Warner CR, Brumley WC, Daniels DH, Joe JrFL, Fazio T. 1986a. Reactions of antioxidants in foods. Food Chem Toxicol 24(10–11):1015-9. DOI: 10.1016/0278-6915(86)90282-6

1029 Warner CR, Daniels DH, Lin FSD, Joe FL, Fazio T. 1986b. Fate of antioxidants and
1030 antioxidant-derived products in deep-fat frying and cookie baking. J Agric Food Chem
1031 34(1):1-5. DOI: 10.1021/jf00067a001
1032 Williams GM, Tanaka T, Maeura Y. 1986. Dose-related inhibition of aflatoxin B1
1033 induced hepatocarcinogenesis by the phenolic antioxidants, butylated hydroxyanisole
1034 and butylated hydroxytoluene. Carcinogenesis 7(7): 1043-50.
1035 Williams GM, Wang CX, Iatropoulos MJ. 1990. Toxicity studies of butylated
1036 hydroxyanisole and butylated hydroxytoluene. II. Chronic feeding studies. Food Chem
1037 Toxicol 28(12):799-806. DOI: 10.1016/0278-6915(90)90052-O
1038 Williams GM, Iatropoulos MJ. 1996. Inhibition of the hepatocarcinogenicity of aflatoxin
1039 B1 in rats by low levels of the phenolic antioxidants butylated hydroxyanisole and
1040 butylated hydroxytoluene. Cancer Lett 104(1):49-53.
1041 Williams GM, Iatropoulos MJ, Whysner J. 1999. Safety assessment of butylated
1042 hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. Food
1043 Chem Toxicol 37(9–10):1027-38. DOI: 10.1016/S0278-6915(99)00085-X
1044 Yamamoto K, Tajima K, Takemura M, Mizutani T. 1991. Further metabolism of 3,5-di-
1045 *tert*-butyl-4-hydroxybenzoic acid, a major metabolite of butylated hydroxytoluene, in
1046 rats. Chem Pharm Bull 39(2):512-4.
1047 Yamamoto K, Fukuda N, Shiroi S, Shiotsuki Y, Nagata Y, Tani T, Sakai T. 1995. Effect
1048 of dietary antioxidants on the susceptibility to hepatic microsomal lipid peroxidation
1049 in the rat. Ann Nutr Metab 39(2):99-106.
1050 Zhang CX, Wu H, Weng XC. 2004. Two novel synthetic antioxidants for deep-frying
1051 oils. Food Chem 84(2):219-22. DOI: 10.1016/S0308-8146(03)00205-X
1052 Zhang YM, Peng Y, Yin XI, Liu ZH, Li G. 2014. Degradation of lignin to BHT by
1053 electrochemical catalysis on Pb/PbO_2 anode in alkaline solutions. J Chem Technol
1054 Biotechnol. DOI: 10.1002/jctb.4282
1055
1056

1057 **Table 1.** 2,6-Di-*tert*-butyl-hydroxytoluene (BHT) and some of its main derived
1058 metabolites.

Abbreviation	Systematic name	Structure	Formula	CAS number
BHT	2,6-Di-*tert*-butyl-hydroxytoluene		$C_{15}H_{24}O$	128-37-0
BHT-CHO	3,5-Di-*tert*-butyl-4-hydroxy-benzaldehyde		$C_{15}H_{22}O_2$	1620-98-0
BHT-COOH	3,5-Di-*tert*-butyl-4-hydroxy-benzoic acid		$C_{15}H_{22}O_3$	1421-49-4
BHT-Q	2,6-Di-*tert*-butyl-2,5-cyclohexadien-1,4-dione		$C_{14}H_{20}O_2$	719-22-2
BHT-QM	2,6-Di-*tert*-butyl-4-methylene-2,5-cyclohexadien-1-one		$C_{15}H_{22}O$	2607-52-5
DBP	2,6-Di-*tert*-butyl-4-phenol		$C_{14}H_{22}O$	128-39-2
BHT-OH	2,6-Di-*tert*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one		$C_{15}H_{24}O_2$	10396-80-2
BHT-CH_2OH	3,5-Di-*tert*-butyl-4-hydroxy-benzyl alcohol		$C_{15}H_{24}O_2$	88-26-6
BHT-OOH	2,6-Di-*tert*-butyl-4-methyl-4-hydroperoxy-2,5-cyclohexadien-1-one		$C_{15}H_{24}O_3$	6485-57-0
TBP	2-*Tert*-butyl-4-methyl-phenol		$C_{11}H_{16}O$	2409-55-4
BHQ	2,6-Di-*tert*-butyl-1,4-benzenediol		$C_{14}H_{22}O_2$	2444-28-2
BHT-OH(t)	3-*tert*-butyl-2-hydroxy-β,β,5-trimethyl-benzeneethanol		$C_{15}H_{24}O_2$	112700-14-8
BHT-OH(t)QM	2-*Tert*-butyl-6-(2-hydroxy-*tert*-butyl)-4-methylene-2,5-cyclohexadien-1-one		$C_{15}H_{22}O_2$	124755-19-7
2-BHT	4,4'-Ethylenebis(2,6-di-*tert*-butyl-phenol)		$C_{30}H_{46}O_2$	1516-94-5

2-BHT-QM (stilbenequinone)	4,4'-Ethanediylidenebis(2,6-di-*tert*-butyl-2,5-cyclohexadien-1-one)		$C_{30}H_{42}O_2$	809-73-4

1059

1060

1061 **Table 2**. Summary of the BHT dietary intake reported in the literature.

Mean estimation (mg/kg bw/day)	**Targeted population** (age)	**Selected BHT containing foods**	**Country** (data from years)	**Reference**
0.13 0.24 0.39	Total (1-65) Adolescents (12-19) Children (1-4)	Instant breakfast, margarine, bread, breakfast cereals, crackers, cakes, cookies, rice, dehydrated potatoes, French-fried potatoes and chips, peanut butter, cooking oils, shortening and salad dressing, puddings, candies, and soft drinks.	Canada (1973)	Kirkpatrick and Lauer 1986
0.075	Total	Potatoes and tuberous plants; cereals and cereal products; fats, oils, mayonnaise, margarine, butter; soups; pastry, cakes, and biscuits; sugar, sweets, and sweet spreads; nuts, seeds, and snacks.	The Netherlands (1987-88)	Verhagen and others 1990
0.125-0.315	Total	Fats from pastry, cake and biscuits; chewing gums; vegetable oils and margarines; fats from pizza, bread, etc.; fats from chips and popcorn; fats from confectionery; fats from other sources.	Italy (1994-96)	Leclercq and others 2000
0.00-0.78	Total	Fats and oils; fat emulsion (water-in-oil); chewing gum; processed fish and fish products including molluscs, crustaceans and echinoderms, and others.	Australia, Brazil, China, Finland, France, Japan, New Zealand, Spain, UK, USA (2000)	JECFA 2000
0.09-0.11	Total	Vegetable oils, fats and creams, margarine, mayonnaise, cereal flakes, grated coconut, chewing gum, coconut milk, and powdered chocolate.	Brazil (1989-2003)	Maziero and others 2001
0.04	Total	Vegetable oils, shortening and margarine; (seasoned) dried, salted and frozen fishery products; mayonnaise; chewing gum; breakfast cereal.	Korea (1998)	Suh and others 2005
0.018-0.025	Students (9 -18)	Fats, sweets, cereals and bread, snacks, nuts, beverages, meat, and fish.	Lebanon (2002-03)	Soubra and others 2007
0.000-0.011 0.000-0.013	Adults (>15) Children (3-14)	Chewing gum.	France (1998-99, 2002-05)	Bemrah and others 2008
0.003-0.022 0.005-0.043 0.007-0.087	Adults (18-64) Adolescents (10-17) Children (3-9)	Fine bakery wares, snacks (dry, savory potato, cereal or starch-based snack products, extruded or expanded savory snack products, other savory snack products and savory peanuts, nuts or hazelnuts), and liquid and solid food supplements.	Europe (22 countries, 1985-2010)	EFSA 2012
0.002-0.221 0.004-0.267	Adults (18-97) Children (1-17)	Concentration data provided by food industry (no further details). Chewing gums not considered.	France, Ireland, Italy, UK (1992-2007)	Vin and others 2013

1062

Table 3. Methodologies employed to determine BHT metabolites in food and related matrixes, together with the abundance data reported.

Matrix	Metabolites	Sample preparation	Analytical technique	Abundance	Reference
Vegetable oil	2-BHT-QM	Extraction with acetonitrile, distillation	TLC, Vis	0-72 µg/L	Leventhal and others 1976
Lard shortening	BHT-Q BHT-CHO BHT-OOH	Preparation of lard with radiolabeled antioxidant	HPLC/MS, GC/MS, TLC	n.a n.a n.a	Warner and others 1986ab
Liquid smoke flavorings	BHT-Q BHT-CHO	Extraction with CH_2Cl_2	GC/MS	n.a. 0.4 mg/L	Guillén and Ibargoitia 1998
Dry fermented sausage	BHT-OH BHT-QM	SDE	GC/MS	0-1884 ng dodecane/g dry matter 0-2635 ng dodecane/g dry matter	Ansorena and others 2001
Drinking water	BHT-CHO	Extraction with $CHCl_3$	GC/MS	n.a.	Brocca and others 2002
River, rain and ground water	BHT-CHO 2-BHT	SPE	GC/MS	0-1627 ng/L n.a.	Fries and Püttmann 2002, 2004
Drinking water	DBP BHT-Q	Purge and trap extraction	GC/MS	0-5000 ng/L 60-600 ng/L	Skjevrak and others 2003
Fluid deep-frying fat	BHT-QM	HS-SPME DVB/CAR/PDMS fiber	GC/MS	$0.36*10^{-6}$ area counts	Goicoechea and others 2008
River and waste water (raw and treated)	BHT-CH_2OH BHT-Q BHT-CHO BHT-COOH	SPE and derivatization with MTBSTFA	GC/MS	0-64 ng/L 0-871 ng/L 13-144 ng/L 13-90 ng/L	Rodil and others 2010
Tap and waste water (raw and treated)	BHT-CH_2OH BHQ BHT-Q BHT-CHO BHT-COOH	SPE	GC/MS	6-78 ng/L 0.1-0.6 ng/L 10-91 ng/L 0-20 ng/L 4-339 ng/L	Rodil and others 2012
Drinking water	BHT-Q	HS-SPME	GC/MS	18-57 µg/L	Lützhøft and others 2013

Abbreviations: DVB/CAR/PDMS: Divinylbenzene/Carboxen/Polydimethylsiloxane; GC: Gas Chromatography; HPLC: High-Performance Liquid Chromatography; HS-SPME: Headspace Solid-Phase Microextraction; MS: Mass Spectrometry; MTBSTFA: N-*tert*-butyldimethylsilyl-N-methyl-trifluoroacetamide; n.a.: not available; SDE: Simultaneous Distillation-Extraction; SPE: Solid-Phase Extraction; TLC: Thin-Layer Chromatography; Vis: Visible spectrophotometry.

FIGURE CAPTIONS

Figure 1. Reaction pathways proposed for antioxidant and prooxidant activity of BHT (Lambert and others 1996; Smirnova and others 2002).

Figure 2. Some of the possible metabolites derived from the biotransformation or degradation of BHT (Matsuo and others 1984; Daniel 1986; Guyton and others 1991; Yamamoto and others 1991; Oikawa and others 1998; Thompson and others 2001; Rodil and others 2010, 2012).

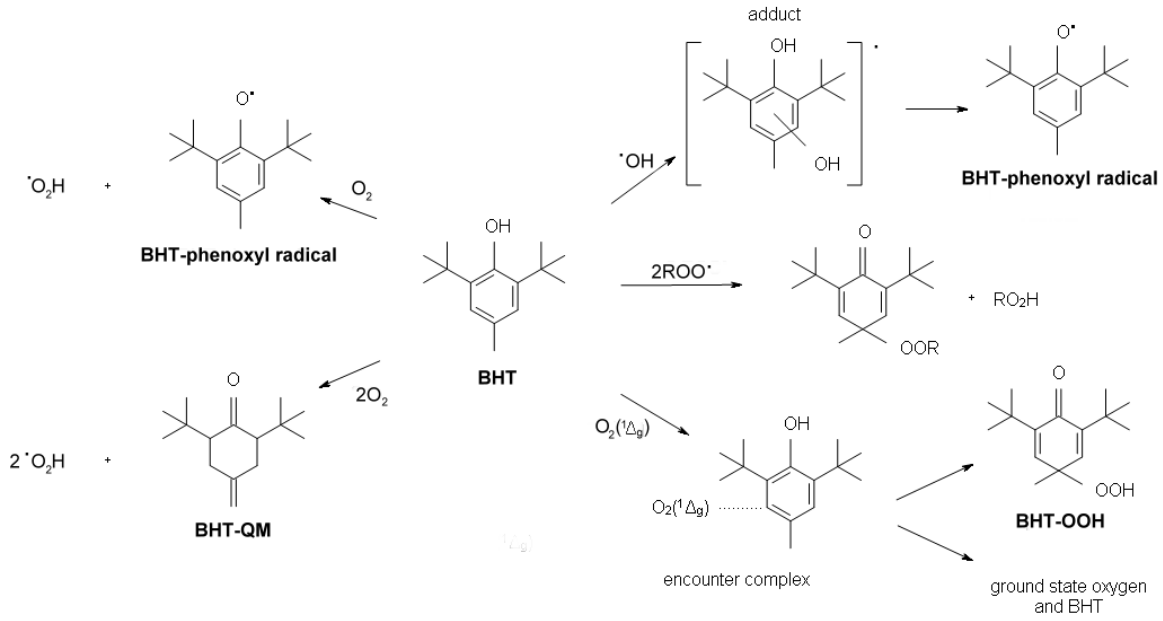
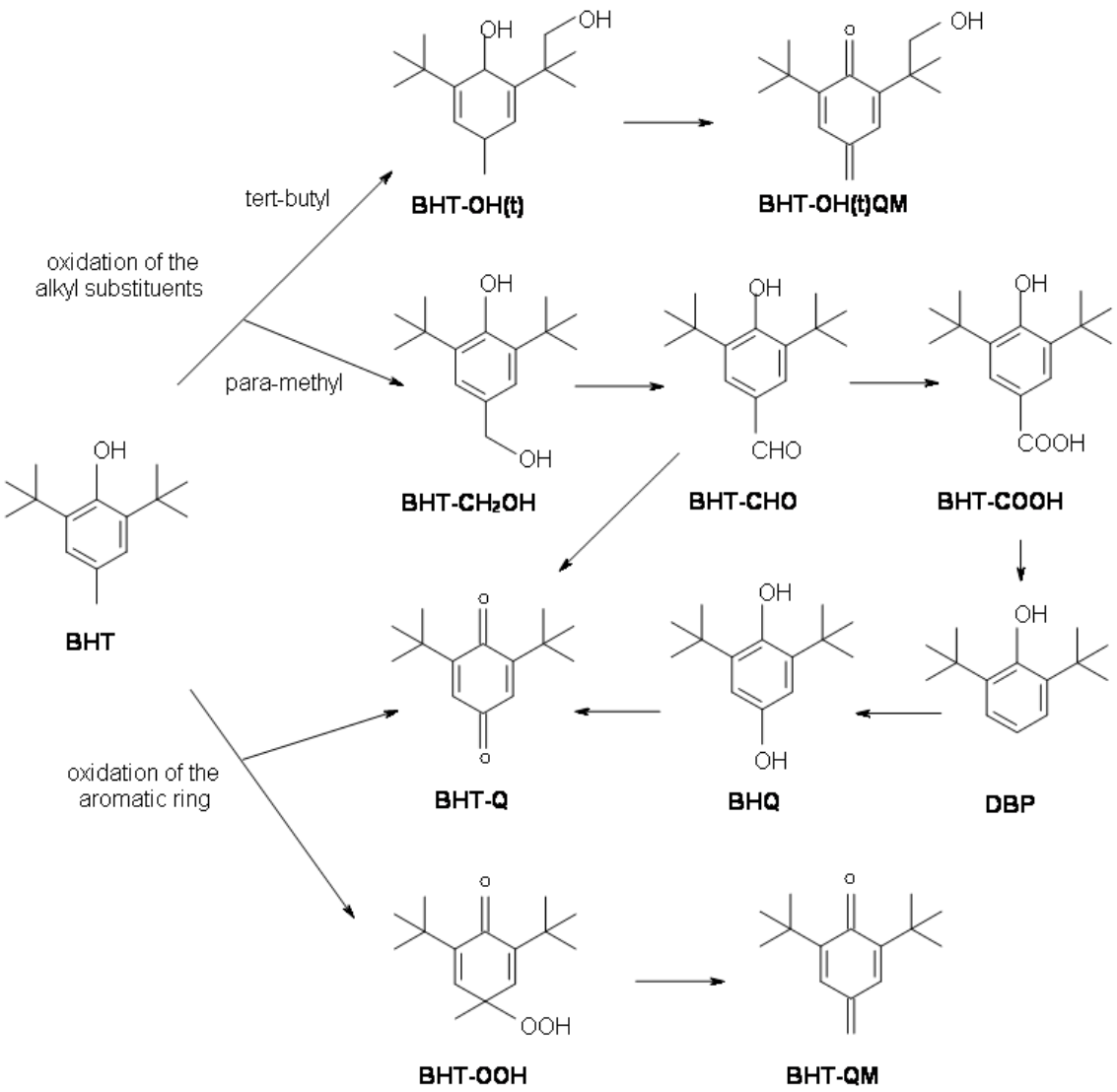

adduct
OH
OH
·OH
O·
BHT-phenoxyl radical
·O2H
+
O2
BHT-phenoxyl radical
OH
BHT
2ROO·
O
OOR
+ RO2H
2O2
O
2 ·O2H
+
BHT-QM
O2(1Δg)
OH
O2(1Δg)
encounter complex
O
OOH
BHT-OOH
ground state oxygen
and BHT
PROOXIDANT REACTIONS
ANTIOXIDANT REACTIONS

Figure 1



1085

1086

1087 **Figure 2**

1088