



Contemporary Review

Butylated hydroxyanisole: Carcinogenic food additive to be avoided or harmless antioxidant important to protect food supply?

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ABSTRACT

Tumor data from rodent bioassays are used for cancer hazard classification with wide-ranging consequences. This paper presents a case study of the synthetic antioxidant butylated hydroxyanisole (BHA), which IARC classified as Group 2B ("possibly carcinogenic to humans") on the basis of forestomach tumors in rodents following chronic dietary exposure to high levels. IARC later determined that the mechanism by which BHA induces forestomach tumors is not relevant to humans; however, the classification has not been revoked. BHA was listed on California Proposition 65 as a direct consequence of the IARC classification, and there is widespread concern among consumers regarding the safety of BHA driven by the perception that it is a carcinogen. While many regulatory agencies have established safe exposure limits for BHA, the IARC classification and Proposition 65 listing resulted in the addition of BHA to lists of substances banned from children's products and products seeking credentials such as EPA's Safer Choice program, as well as mandatory product labeling. Classifications have consequences that many times pre-empt the ability to conduct an exposure-based risk-based assessment. It is imperative to consider human relevance of both the endpoint and exposure conditions as fundamental to hazard identification.

1. Cancer hazard classification: historical perspectives and implications

Our focus on identifying carcinogens started in the 1700s when we first began to understand cancer as a disease associated with occupations, lifestyle or exposures. Our use of experimental animal models to identify chemical carcinogens started in the early 1900s (e.g., Yamagiwa and Ichikawa, 1918; Hueper et al., 1938) and thus began our efforts to identify carcinogenic hazards with the intent of minimizing exposure to protect human health. The International Agency for Research on Cancer (IARC) was established in 1965 and initiated a program to classify substances according to their potential to cause cancer. In the years that followed, many regulatory bodies around the world started their own system to classify chemicals, and in most cases the classification was focused on 'hazard identification' without any context of human exposure conditions required for the induction of cancer. These classification schemes continue to be used today although it is now clear that this overly simplistic definition is neither valid nor helpful, and are not suitable for nongenotoxic rodent carcinogens (e.g., Bevan and Harrison, 2017; Boobis et al., 2016).

Some chemicals raise a concern for carcinogenicity following any route of exposure and at very low doses that might not require chronic exposure. For others, however, carcinogenicity is route-specific and requires chronic exposure to doses that exceed a biological threshold. Thus the identification of a carcinogenic hazard should not be separated from the context of exposure, including route, magnitude, and duration. A recent example that the public generally recognizes is the 2015 classification by IARC of processed meat as carcinogenic to humans (Group 1), based on sufficient evidence in humans that the consumption of processed meat causes colorectal cancer. This classification does not (nor was it intended to) raise a concern for occasional and intentional consumption of processed meat. The list of IARC Group 1 (known human) carcinogens does include potent contaminants like aflatoxins, for which significant efforts (including strict regulations) are made to ensure human exposure is kept as low as possible. However, this list also includes intentionally ingested substances (e.g., processed meat, salted fish, ethanol in alcoholic beverages), and other scenarios that raise a concern for human health at sufficiently high exposures, but likely pose negligible risk at sufficiently low exposures or acute/occasional higher exposures. The situation is even more complicated when one looks at the

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list of IARC Group 2B (“possible”) carcinogens, which includes substances like caffeic acid and β -myrcene, which are naturally occurring substances found widespread throughout a healthy diet, are not considered by any regulatory agency to raise a concern for human health, and may in fact offer health benefits (e.g., Espíndola et al., 2019; Felter et al., 2020). Thus, the listing of a substance as a carcinogen (whether by IARC or any other agency) is not informative as to whether there is an actual concern for human health under relevant conditions of exposure, and what those conditions are. While this is intended to be addressed by risk assessment, it remains the case that there are many downstream consequences from classification alone, such that a chemical classified as a carcinogen is subject to significant regulation, bans, and consumer misperception, which in many cases are unfounded. This manuscript presents a case study of butylated hydroxyanisole (BHA), including a summary of the data that led to its listing as a 2B human carcinogen by IARC and classification by others including California Proposition 65, and the downstream consequences of this listing.

2. Case study: butylated hydroxyanisole (CAS# 25013-16-5)

2.1. What is BHA, how is it used, where can it be found?

Butylated hydroxyanisole (BHA) is a synthetic mixture of the isomers 3-tert-butyl-4-hydroxyanisole (3-BHA) and 2-tert-butyl-4-hydroxyanisole (2-BHA) (Fig. 1). The purity of BHA is specified to be not less than 85% 3-BHA isomer in the mixture according to EU legislation and JECFA (EFSA, 2011).

BHA is one of many synthetic antioxidants widely used in different product categories including food, food packaging, pharmaceuticals and cosmetics. Since 1947, BHA has been added to edible fats and fat-containing foods as an antioxidant (Lam et al., 1979). It serves an important role in preserving food by preventing oxidative rancidity of fats and oils as well as preventing the loss of activity of oil-soluble vitamins. In 1984, the International Life Sciences Institute indicated that approximately 50 countries allowed the use of BHA as a food additive (ILSI, 1984). Today it can still be found in many foods with the maximum permitted level (MPL) ranging from 2 ppm to 5000 ppm in different food types and across different regions (21CFR172.110; EFSA, 2011; Davoli et al., 2017; Health Canada, 2020). In addition to its use as a direct food additive, BHA is added to packaging materials in order to provide protection to foods inside the package through the volatilization of the antioxidant (Shahidi and Zhong, 2005).

2.2. Human exposure to BHA

The major route of human exposure to BHA is food ingestion. The daily dietary intake of BHA has been estimated in many countries over the years. There are several methods that have been used to estimate the intake of BHA including estimation based on poundage data, household economic surveys and sales data, model diets, individual dietary records, General Standard for Food Additives (GSFA) or MPLs (WHO, 1999; EFSA, 2011). The mean daily intake of BHA varies from about 0.002 mg/kg/day to 0.3 mg/kg/day by consumer ages, regions and estimate methods (Freydberg and Gortner, 1982; Ishiwata et al., 2003; Maziero et al., 2001; Suh et al., 2005; Environment Canada HC, 2010;

EFSA, 2011). In addition to its use in food, exposure to BHA also occurs from its use as an antioxidant in pharmaceuticals and cosmetic products (IARC 1986; CIR 2006). The maximum estimated intake of BHA from personal care products in Canada was reported to be 0.06–0.07 mg/kg/day (Environment Canada HC, 2010).

2.3. Toxicology of BHA

2.3.1. Genotoxicity and mutagenicity

The substantial database on the genetic toxicology of BHA has been critically reviewed elsewhere (ECHA, 2020; EFSA, 2011; Environment Canada HC, 2010; IARC, 1986; JECFA, 1982; 1987; Whysner and Williams, 1996). Overall, BHA is not mutagenic in *Salmonella* with or without S9 metabolic activation, nor is BHA mutagenic in mammalian test systems *in vitro* when tested at the HGPRT locus in rat liver cells or Chinese hamster ovary cells without S9 metabolic activation.

DNA binding studies conducted on the rodent forestomach provide important mechanistic and risk information since this is the site of tumor induction following chronic oral administration of BHA (see Section 2.3.3). Ito et al. (1991) found no evidence of DNA adducts in the forestomach of rats exposed to 1000 mg/kg BHA for 5 days. Saito et al. (1989) exposed F344 rats to 1000 mg/kg 3-tert-BHA and two of its metabolites via oral gavage for either 1 or 5 days, none of which led to the formation of DNA adducts via ^{32}P -postlabeling (Saito et al., 1989).

Overall, the weight of evidence indicates that BHA does not pose a genotoxicity hazard, a conclusion agreed upon by multiple authoritative bodies (EFSA, 2011; Health Environment Canada and Health Canada, 2010; IARC, 1986; SCF, 1989; WHO/FAO JECFA, 1986).

2.3.2. Subchronic toxicity

Several subchronic feeding studies with BHA have been critically reviewed elsewhere (EFSA, 2011; WHO/FAO JECFA, 1986; 1989; SCF, 1989). Most of these studies were designed with limited endpoints, either as range-finding studies for subsequent chronic studies, or to assess the dose-response and time-course for forestomach lesions specifically (as described in Section 2.3.3, the rodent forestomach is the primary target organ in longer-term BHA studies in rodents). Collectively, these studies indicate that subacute (28–30 day) and subchronic (≤ 91 day) exposure to BHA increases epithelial damage, hyperplasia, and inflammation in the rodent forestomach. Consistent with tissue damage and hyperplastic responses, BHA also increases cell proliferation as measured by labelling index.

While data in non-rodent species are limited, they provide further support that lesions associated with oral BHA exposure to large doses are largely confined to the portal of entry, with a degree of susceptibility or sensitivity less than that of the rodent forestomach (Würten and Olsen, 1986; Ikeda et al., 1986; Tobe et al., 1986). Most relevant to human health, female cynomolgus monkeys were exposed to 0, 125, or 500 mg/kg BHA via corn oil gavage 5 days per week for 12 weeks (note: the 500 mg/kg dose was lowered to 250 mg/kg after 4 weeks due to vomiting). At study termination, no gross or histopathological lesions were observed that were considered treatment-related (Iverson et al., 1986). The mitotic index was increased ~2-fold in the esophagus of the high dose animals; however, the study authors noted that this was likely due to the high concentrations of BHA in the gavage solution (250 mg/mL) and the deliberate administration of BHA to the distal esophagus. Collectively, data from subchronic studies indicate that toxic effects of BHA are limited to the rodent forestomach, and that BHA is generally non-toxic to species lacking a forestomach.

2.3.3. Carcinogenicity

The carcinogenicity database for BHA has also been critically reviewed in depth (EFSA, 2011; IARC, 1986, 2003; Health Environment Canada and Health Canada, 2010; JECFA, 1989; NTP, 2016; SCF, 1989; Whysner and Williams, 1996), and no additional relevant toxicity studies have been published since these reviews. The multi-dose BHA

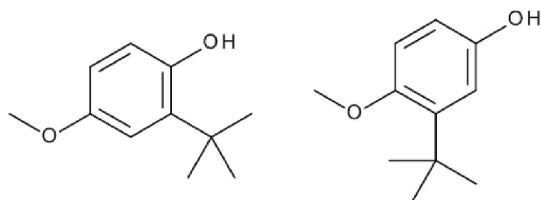


Fig. 1. Structure of 3-tert-butyl-4-hydroxyanisole (left) and 2-tert-butyl-4-hydroxyanisole (right).

carcinogenicity studies are summarized in Table 1. The rodent forestomach is the only tissue reported to develop tumors following chronic oral exposure of BHA. Tumors for other organs were also evaluated in rats exposed to BHA and their incidences are similar to those in controls (Ito et al. 1983, 1986b). Although guinea pigs are sometimes classified as rodents, they are distinct from rodents (D'Erchia et al., 1996); they do not have a forestomach and do not develop lesions in the glandular stomach when fed a diet containing 1% BHA for 20 months (Ito and Hirose 1987).

The dose-response for BHA-induced forestomach tumors was best demonstrated in Ito et al. (1986a), where male F344 rats (50/group) were exposed to 0, 55, 110, 230, 428, and 1323 mg/kg/day BHA in diet for two years. At study termination, there was a dose-dependent increase in forestomach hyperplasia starting with a 2% incidence at the lowest dose increasing to 100% in the highest dose group (Table 2). Benign papillomas were observed in the two highest doses, but squamous cell carcinomas were limited to the highest treatment group. No other treatment-related tumors were observed.

It is also notable that even within a species, there is a significant difference in response to dietary administration of BHA. Tamano et al. (1998) evaluated the forestomach carcinogenicity of BHA in male rats from four different strains: F344, SHR, Lewis, and Sprague-Dawley. Groups of 30 male rats per strain were administered basal diet or a diet containing 2% BHA. Squamous cell papillomas and hyperplasia were found in the forestomach of rats from each strain, but the incidences of squamous cell carcinoma differed considerably: 76.7% (23/30) in SHR rats; 36.7% (11/30) in SD rats; 26.7% (8/30) in F344 rats; and 6.7% (2/30) in Lewis rats.

In summary, dietary exposure to BHA causes forestomach papillomas and squamous cell carcinomas in rodents (rats, mice, and hamsters) with dose and temporal trends as well as differences in sensitivity across different strains (D'Erchia et al., 1996). Importantly, the absence of carcinogenicity in mammals that lack a forestomach lends additional support for the lack of human relevance of BHA-induced forestomach tumors. For human health risk assessment, the lack of carcinogenicity in the glandular stomach and esophagus of all species studied indicates a lack of potential carcinogenicity.

Table 2

Dose dependency of BHA-Induced forestomach lesions (Ito et al., 1986a).

Dose (mg/kg/day)	Concentration in food (%)	Hyperplasia	Papillomas	Squamous Cell Carcinomas
0	0	0/50	0/50	0/50
55	0.125	1/50	0/50	0/50
110	0.25	7/50	0/50	0/50
230	0.5	16/50	0/50	0/50
428	1	44/50	10/50	0/50
1323	2	50/50	50/50	11/50

2.3.4. Epidemiological data

Although BHA is found widely in the diet at low levels, there are very limited epidemiological data to evaluate the relationship between human cancer and exposure specifically to BHA. In the only identified study, no increase in cancer risk at typical dietary intake levels was identified from a population-based nested case-control study of stomach cancer in men and women within the Netherlands Cohort Study (Botterweck et al., 2000). While a lack of complete data on the ingestion of BHA limits the usefulness of this study, it is interesting that a statistically non-significant decrease in stomach cancer risk was observed with increasing BHA intake based on a rate ratio (RR) highest/lowest intake of BHA of 0.57 (95% confidence interval: 0.25–1.30). This was also true for the antioxidant butylated hydroxytoluene (BHT), where the RR was determined to be 0.74 (95% confidence interval: 0.38–1.43). The authors state that, “The finding of an apparent decreased risk with increasing BHA or BHT intake seem to be more in line with the hypothesis that synthetic antioxidants may be protective for cancer and supports findings of studies suggesting that low doses of BHA and BHT inhibit carcinogenesis (Hocman, 1988; Wattenberg, 1986; Williams et al., 1986, 1999). Anti-oxidants such as BHA and BHT may have beneficial effects by protecting against toxic compounds that are derived from the destruction of nutrients and the oxidation of fatty acids in foods (Grice et al., 1986).”

Table 1Carcinogenicity studies on BHA: Incidence of papillomas and squamous cell carcinomas of the forestomach^a.

Species	Dose, % in diet (mg/kg/day)	Incidence of Forestomach Papillomas	Incidence of Forestomach Squamous Cell Carcinomas	Reference
Syrian gold hamster, M	0, 1, 2% in diet (0, 1200, 2400 mg/kg/day) ^b	2/12, 12/13, 4/4	0/12, 1/13, 0/4	Ito et al. (1986b)
Syrian gold hamster	0, 1, 2% in diet (0, 1200, 2400 mg/kg/day) ^b	0/52, 54/55, 38/40	0/52, 4/55, 4/40	Masui et al. (1986)
B6C3F1 mice, M	0, 0.5, 1% in diet 0, 750, 1500 mg/kg/day) ^b	0/16, 0/21, 1/22	0/16, 0/21, 0/22	Ito et al. (1986b)
B6C3F1 mice	0, 0.5, 1% in diet (0, 750, 1500 mg/kg/day) ^b	0/39, 5/37, 5/43	0/39, 1/37, 2/43	Masui et al. (1986)
F344 rats, M	0, 0.5, 2% in diet (0, 98, 414 mg/kg/day)	0/51, 1/50, 52/52	0/51, 0/50, 18/52	Ito et al. (1983)
F344 rats, F	0, 0.5, 2% in diet (0, 108, 474 mg/kg/day)	0/51, 1/51, 49/51	0/51, 0/51, 15/51	Ito et al. (1983)
F344 rats, M	0, 1, 2% in diet (0, 450, 900 mg/kg/day) ^b	0/23, 21/25, 26/26	0/23, 0/25, 9/26	Ito et al. (1986b)
F344 rats, M	0, 0.125, 0.25, 0.5, 1, 2% in diet (0, 55, 110, 230, 428, 1323 mg/kg/day)	0/50, 0/50, 0/50, 0/50, 10/50, 50/50	0/50, 0/50, 0/50, 0/50, 0/50, 11/50	Ito et al. (1986a)
F344 rats, M	0, 1, 2% in diet (0, 450, 900 mg/kg/day) ^b	0/92, 71/94, 86/94	0/92, 0/94, 13/94	Masui et al. (1986)
F344 rats, M	0, 0.4% in diet (0, 180 mg/kg/day) ^b	0/25, 0/26	0/25, 0/26	Hirose et al. (1997)
F344 rats	0, 2% in diet (0, 974 mg/kg/day)	1/30, 30/30	0/30, 8/30	Tamano et al. (1998)
SHR rats	0, 2% in diet (0, 1375 mg/kg/day)	0/29, 30/30	0/29, 23/30	Tamano et al. (1998)
SD rats	0, 2% in diet (0, 956 mg/kg/day)	0/30, 30/30	0/30, 11/30	Tamano et al. (1998)
Lewis rats	0, 2% in diet (0, 1041 mg/kg/day)	0/30, 30/30	0/30, 2/30	Tamano et al. (1998)

^a All studies were 104 week duration; dose conversions as reported by authors unless otherwise specified.

^b Dose conversion based on EFSA (2011).

2.4. Mode of action (MOA) for BHA-induced forestomach tumors

Understanding the MOA for chemically-induced rodent tumors is important for hazard identification and determining relevance to human health as well as informing the most appropriate method for quantitative risk assessment (US EPA, 2005). A wealth of data exists for BHA as summarized briefly below.

2.4.1. Traditional data streams

The MOA for forestomach tumors induced in rodents following chronic administration of BHA has been described in detail by Whysner and Williams (1996). BHA is not DNA-reactive, and repeat-dose studies in rodents have confirmed that it is cytotoxic and increases cell proliferation at the site of contact following administration at high doses, with the rodent forestomach being particularly susceptible. These effects are largely reversible, and thresholds have been demonstrated by many investigators as demonstrated by:

- A 9 or 27-day exposure of up to 2% BHA in the diet resulted in a marked increase in the labeling index in the prefundic region of the forestomach of male F344 rats (Nera et al., 1984; Clayson et al., 1986). A no effect level of 0.25% was established based on the thymidine labeling index.
- Iverson et al. (1985) fed male F344 rats a diet containing concentrations of 0, 0.1, 0.25, 0.5 or 2% BHA for 13 weeks followed by a recovery period where the rats received a basal diet for up to 9 weeks. At 13 weeks, the [methyl-3H]thymidine labeling index was raised in the two highest dose groups only, with an apparent no effect level at 0.25%; these rapidly returned to near control values within one week after withdrawal of BHA. Proliferative lesions in the forestomach epithelium were only seen in the highest dose (2%) rats.
- Nera et al. (1988) exposed male F344 rats to a diet containing 2% BHA for 0, 3, 6, 12 or 24 months, with those rats given BHA for the shorter time periods then switched to the basal diet for the remainder of the 24 month study period. An increased rate of cell proliferation was seen as long as BHA was administered but was largely reversible even following 12 months of exposure. Forestomach squamous cell carcinomas were only seen in rats exposed to BHA for 12 or 24 months. It is also noteworthy that Nera et al. (1988) reported that the rats given a diet with 2% BHA consumed approximately 20% less food than those given the basal diet, and their body weight was also about 20% less than that of controls. Thus, the authors concluded that 2% BHA in the diet exceeds the Maximum Tolerated Dose for rats.

In summary, these studies (supported by many others summarized by Whysner and Williams, 1996) consistently demonstrate a dose-dependent progression of sequelae in the rat forestomach from hyperplasia to papillomas to squamous cell carcinomas. A clear threshold for the induction of cell proliferation has been demonstrated, with no effects at 0.25% in the diet. Further, complete reversibility was found following exposure of rats for 6 months, and partial reversibility following exposure as long as 12 months. An increase in carcinomas was seen only following chronic exposure to high levels of BHA.

2.4.2. AOP (Adverse Outcome Pathway) for forestomach tumors

Since there is strong evidence linking BHA-induced cytotoxicity to hyperplasia that leads to carcinogenicity (Cantoreggi et al., 1993; Whysner and Williams, 1996), Proctor et al. developed an Adverse Outcome Pathway (AOP) framework for forestomach tumors induced by non-genotoxic initiating events (Proctor et al., 2018). In this framework, the initiating event (IE, cellular level) for BHA is cytotoxicity which can be supported by findings on mucosal injury, forestomach edema, erosion, and ulceration induced by bolus doses. The key event 1 (KE1, cellular level) is cell proliferation, and the key event 2 (KE2, tissue level) is hyperplasia. The adverse outcome (AO, organism level) for BHA is

squamous cell papillomas and carcinomas which were observed in forestomach of rats, hamsters, and mice in multiple chronic studies (Tables 1 and 2). There are also data suggesting that the cytotoxicity of BHA might be mediated through GSH depletion and/or protein binding. Therefore, GSH depletion may serve as the pre-MIE (molecular initiation event) for BHA (Shibata et al., 1993).

2.4.3. Mode of action: summary

A wealth of data on BHA provides robust evidence for BHA to induce forestomach tumors in rodents following chronic exposure to high dietary concentrations that cause cytotoxicity and an increase in cell proliferation. There is a clear biological basis and empirical data to support a threshold for both the noncancer effects and the tumors that arise as a secondary consequence. There are, in fact, a number of studies that suggest low levels of BHA may play a role in protecting against cancer; this is consistent with its properties as an antioxidant (see section 3.0 below).

2.5. Cancer classifications of BHA

Based on the results of chronic rodent studies, BHA has been classified as a carcinogen by several regulatory/advisory bodies including IARC, the State of California, and NTP's Report on Carcinogens; industry self-classifications have also been published by ECHA:

- **IARC (International Agency for Research on Cancer):** IARC reviewed BHA in October 1985 and classified it in Group 2B (Possible Human Carcinogen) based on "sufficient evidence of carcinogenicity in experimental animals" and "no data were available on the carcinogenicity" in human beings (IARC, 1987). IARC acknowledged that BHA is not genotoxic in bacterial and mammalian assays and further acknowledged that when tested in combination with other chemicals (usually known mutagens or carcinogens), BHA reduced the activity of other mutagens/carcinogens (IARC, 1986). It is noteworthy that subsequent to its classification of BHA as a possible human carcinogen, IARC held a workshop in which they discussed the mechanism by which BHA induces forestomach tumors in rats and determined that the mechanism was not relevant to humans (IARC, 2003); however, the classification of BHA has not been reconsidered and it remains classified by IARC in Group 2B.
- **California Proposition 65:** BHA was listed on January 1, 1990 as a chemical known to the State to cause cancer under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65); a No Significant Risk Level of 4 mg/day was established (OEHHA, 1990).
- **NTP Report on Carcinogens (RoC):** BHA was first listed in the Sixth Annual RoC (NTP, 1991) and recently the Fourteenth Edition (NTP, 2016) as "reasonably anticipated to be a human carcinogen" based on sufficient evidence of carcinogenicity from studies in experimental animals. In the Fourteenth Edition, NTP RoC acknowledged that the epidemiological study of stomach cancer in men and women within the Netherlands showed no increase in risk at typical levels of dietary intake of BHA (Botterweck et al., 2000).
- **ECHA (European Chemicals Agency):** ECHA has not issued a harmonized classification for BHA. While the majority of registrants self-classified BHA as Carc 2 (Hazard statement, H351: Suspected of causing cancer) as per the Classification, Labelling and Packaging (CLP) regulation ((EC) No 1272/2008); other companies indicated a classification of Carc 1B (Hazard Statement H350: May cause cancer) (<https://echa.europa.eu/information-on-chemicals/cl-inventories-database/-/discli/details/97532>).

3. BHA as an antioxidant

While the focus on BHA as a food additive has been on repeat-dose toxicology studies and the determination of a safe intake level, it is important to recognize that there are several studies documenting

potential health benefits associated with low levels of BHA. Antioxidants, both endogenous and exogenous (i.e., dietary) play an important role in human health by inhibiting the propagation of reactive oxygen species and other free radicals that are constantly formed during normal physiological processes and have been implicated in the etiology of a number of human diseases including cancer. Dietary antioxidants are both naturally-occurring in our food (e.g., flavonoids, arginine, vitamin C, vitamin E, carotenoids, resveratrol, selenium), and are also commonly added to food to inhibit oxidative processes that lead to spoilage. Antioxidants that are intentionally added include naturally-occurring substances and also synthetic antioxidants such as BHA. There is no basis to conclude that the status of an antioxidant as being “natural” or “synthetic” has any relevance to safety, and it is noted that synthetic antioxidants are often added to food because they are designed to be effective at very low concentrations.

As described in this manuscript, chronic high doses of BHA have been shown to cause forestomach tumors in rodents. These studies resulted in BHA being classified as a carcinogen, while it is recognized that there is no health concern for the levels in our diet. In fact, there is some evidence to suggest a health-protective role for low levels of BHA, mediated via its antioxidant properties (reviewed in IARC, 1986; JECFA, 1986, 1987; 1989; Whysner and Williams, 1996). While BHA alone does not affect the tumor incidence (higher or lower) in organs other than forestomach in rats (Ito et al. 1983, 1986b), Hocman (1988) provides an overview of several studies that demonstrated an inhibitory effect of BHA or BHT on the incidence of tumors in several target tissues induced by a variety of chemical carcinogens. Both BHA and BHT showed a decrease in the number of animals with tumors and the number of tumors per animal, as well as a longer latency period. For example, Williams et al. (1986) reported that 63% of male F344 rats administered aflatoxin in the diet developed hepatocellular neoplasms; this incidence dropped to only 13% of rats also given 1000 ppm (0.1%) BHA and 8% of rats given 6000 ppm (0.6%) BHA. Hocman concludes, “In spite of the possible tumor-promoting properties [of BHA and BHT] they could not be considered overtly toxic. Their pronounced chemoprotective role against some forms of chemical carcinogenesis deserves considerable attention.”

Indeed, chronic high doses of other antioxidants have also been shown to cause adverse health effects including cancer in laboratory animals, and many publications have addressed the fact that chemicals that act as antioxidants at low doses may act as pro-oxidants at higher doses (Saeidnia and Abdollahi, 2013; Mennen et al., 2005; Skibola and Smith, 2000; Maurya and Devasagayam, 2010). Notably, caffeic acid is a naturally occurring antioxidant found in all plants and thus widespread in our diet. It has been shown to both induce and inhibit carcinogenesis in rodents as a function of dose (Hirose et al., 1997). Similar to BHA, caffeic acid is listed on California Proposition 65 as a carcinogen based on the IARC classification in Group 2B (possible human carcinogen), largely on the basis of rodent forestomach tumors following chronic administration to high doses. IARC (1993) does acknowledge that “Oral administration of caffeic acid in combination with known carcinogens resulted in enhancing or inhibiting effects depending upon the carcinogen and the time of administration” but went on to classify caffeic acid in Group 2B, which subsequently led to the listing on California Proposition 65 under the Authoritative Body mechanism. Meanwhile, no food safety agency has raised any concern regarding our daily ingestion of caffeic acid.

In addition to the many examples of rodent carcinogens that pose no or negligible concern for human health under certain exposure conditions, there are also many examples in humans that illustrate this point. Sunlight is a known human carcinogen, but is well-recognized as playing an important role in human health. High levels of Vitamin A were found to increase the risk of lung cancer in a controlled clinical trial (Goodman et al., 2004), but low levels are necessary for human health. Coffee is known to contain at least 21 rodent carcinogens (Ames and Gold, 2000), yet has been shown to have many health benefits while posing no risk for

human cancer (IARC International Agency for Research on Cancer, 2018; OEHHA, 2019). Bacon was classified by IARC as a known human carcinogen (IARC, 2015), yet does not raise either an objective or perceived safety concern for consumption in moderation.

4. Risk/safety assessment and regulatory opinions of BHA

There are a number of questions that should be considered in conducting a quantitative risk assessment for BHA: (1) are effects in the rodent forestomach relevant to humans and an appropriate basis for the risk assessment? (2) if the safety assessment is based on effects in the rodent forestomach, what is the appropriate dose metric given that the effect is at the site of contact (concentration in the diet or mg/kg/day)? (3) are there any other adverse health effects that should be considered in the risk assessment? (4) how should we take into account studies that suggest a health protective role for low levels of BHA that are reflective of consumer exposures (versus the high concentrations used in the rodent cancer bioassays)?

The safety of BHA for use as an antioxidant in our food supply has been confirmed (and reconfirmed over many years) by both regulators (e.g., FDA, EFSA, Health Canada, JECFA) and research scientists (e.g., Whysner and Williams, 1996). Limits of BHA in many food types have been established accordingly. While it is not the goal of this manuscript to conduct an in-depth safety assessment of BHA, a short synopsis of major regulatory decisions is helpful as we consider the topic of this manuscript – is BHA a carcinogen to be avoided, or a potentially health-protective antioxidant?

Joint FAO/WHO Expert Committee on Food Additives (JECFA): JECFA first evaluated the safety of BHA as a food additive in 1961, with many subsequent evaluations through the 1980s. In the most recent assessment conducted in 1988, JECFA stated that “the Committee believed that the relevance to human beings of the rat studies, while inherently questionable because the target tissue in the rat has no human counterpart, could not readily be ignored. The Committee concluded that an ADI could be established on the basis of the dose-dependence and reversibility of the lesions produced in the rat...” An Acceptable Daily Intake (ADI) for BHA of 0.5 mg/kg/day was established on this based on a NOAEL of 0.1% BHA in the diet (equivalent to an intake of 50 mg/kg/day) in long-term studies in the rat (JECFA, 1989).

European Food Safety Authority (EFSA): BHA is authorized according to Directive 95/2/EC2 as a food additive (antioxidant) with maximum levels established for different food categories. EFSA (2011) conducted an updated safety assessment of BHA, including an updated quantitative analysis to calculate a benchmark dose (BMD) for effects in the rat forestomach. However, the Panel also “considered that forestomach hyperplasia in rodents may no longer be relevant for human risk assessment.” EFSA (2011) established an ADI of 1.0 mg/kg/day based on a NOAEL of 0.125% BHA in the diet (100 mg/kg/day) for reproductive and developmental toxicity in Sprague-Dawley rats exposed to BHA before mating, during gestation and through to 90 days of postnatal age (Vorhees et al., 1981). The ADI was calculated by applying an uncertainty factor of 100 to the NOAEL of 100 mg/kg/day. Although the ADI is no longer based on forestomach effects and EFSA has questioned the human relevance of those effects, EFSA did also note that the NOAEL of 100 mg/kg/day also covers the BMDL10 values for forestomach hyperplasia observed in the rat. EFSA (2011) conducted updated exposure assessments for BHA and concluded that both average and high level intakes for children and adults are unlikely to exceed the ADI of 1.0 mg/kg/day such that there is no safety concern.

US FDA: The FDA has studied the use of BHA in foods for decades and has determined that at the current usage levels, BHA does not pose safety concerns. BHA is generally recognized as safe for use in food when the total content of antioxidants is not over 0.02% of fat or oil content, including essential (volatile) oil content of food (21CFR182.3169). For specified food like beverages and desserts prepared from dry mixes, BHA

level is limited to 2 ppm, for other food such as active dry yeast, this level can go up to 1000 ppm (21CFR172.110). FDA does require food companies to list BHA as an additive, on product labels.

Health Canada: Health Canada used a margin of exposure (MOE) approach to evaluate the health risk for BHA in human both cancer and non-cancer endpoints. For the cancer endpoint, Health Canada compared a NOEL of 55 mg/kg/day for hyperplasia and a NOAEL of 230 mg/kg/day for neoplasia in rat forestomach (Ito et al., 1986) with the highest mean BHA exposure of 0.095 mg/kg/day in children 4–8 years old which results in an MOE greater than 500. Health Canada considered that this MOE would be adequate to protect human health from a cancer endpoint. Similarly, for the non-cancer endpoint, Health Canada compared the lowest critical non-neoplastic effect level secondary to induction of UDP-glucuronyl transferase in the liver and small intestine (50 mg/kg/day) with the highest mean BHA exposure of 0.095 mg/kg/day in 4- to 8 years old children which results in an MOE greater than 500. Since the LOEL is based on a threshold for induction of a compensatory liver enzyme, a non-severe adverse effect, this MOE would be considered adequate to protect human health for non-cancer effects as well (Environment Canada HC, 2010).

5. Consequences of classification

While there is no human health concern for intake of BHA under current exposure conditions, the rodent forestomach tumors have resulted in the classification of BHA as a carcinogen by many regulatory/advisory bodies and this has resulted in a number of downstream consequences for both food and other consumer products.

5.1. Public perception of BHA

Public perception of the safety of food additives is significantly influenced by information readily available to them on the internet, and many organizations have raised questions or outright impugned the safety of BHA. The Environmental Working Group (EWG, 2014) has included BHA in a group of food additives they call the “Dirty Dozen” with a tag line of “GENERALLY RECOGNIZED AS SAFE, BUT IS IT?” EWG recognizes that questions are raised about cancer and endocrine disruption, ending with a recommendation that consumers “Look for BHA on product labels and avoid it.”¹ The EWG listing has been cited by many others including the Food Babe,² who has included it in her list of ingredients to avoid, and includes a list of foods where it can be found. BHA is listed on the website “Stop Killing My Kids” where it is described (along with BHT) as being “dangerous to our children’s health” and our ability to rely on the FDA to protect consumer health is questioned.³ Many similar websites are found on the internet, most of which raise an alarm based on classification of BHA as a carcinogen. This goes beyond food to also include websites that raise concern for BHA in cosmetics (e. g., Campaign for Safe Cosmetics).⁴

5.2. Consequences beyond food

While this manuscript has focused on BHA as an antioxidant in food, it is also important to recognize that the consequences of classifying BHA as a carcinogen has repercussions in many other sectors. For example, a hazard-based (vs risk- or safety-based) approach to evaluating chemicals

has also resulted in BHA being listed as a chemical of concern by some U. S. states, including Oregon and New York. The Oregon Health Authority listed BHA as a high priority chemical of concern for children’s health when used in children’s products,⁵ regardless of the level or potential for actual exposure. In February 2020, New York state passed a law requiring disclosure of “chemicals of concern” in children’s products and allowing the state to determine a set of high-priority substances that could face bans.⁶ It was determined that, among other health endpoints, a “chemical may be listed as a chemical of concern if it has been identified by a government entity and/or identified on the basis of credible scientific evidence as being: (i) a carcinogen, reproductive or developmental toxicant, neurotoxicant, asthmagin, or endocrine disruptor.” BHA is on this list. Additional consequences include mandatory labeling of cleaning products that contain BHA as a result of new California legislation (California Cleaning Products Right to Know Act, SB 258). Similarly, a number of credentialing programs prohibit the use of any ingredients classified as carcinogens. For example, the Safer Choice program administered by the EPA is a hazard-based system designed to “help consumers, businesses, and purchasers find products that perform and contain ingredients that are safer for human health and the environment.”⁷ It requires that all ingredients seeking the Safer Choice seal be screened for chemicals that might present a serious concern for human health or the environment. Accordingly, the presence of an ingredient classified as a carcinogen will generally mean that the product would not be granted a Safer Choice label. While this likely resonates with consumers who see carcinogens as dangerous substances to be avoided, it is clearly complicated by chemicals that only pose a safety concern at very high doses such that its presence at a low level in a consumer product poses no such concern.

6. Conclusion

Significant effort is expended by both industry and regulators to ensure that our food is safe, including all food additives. For some endpoints such as carcinogenicity, the situation is made more complicated by laws that prohibit the use of a substance classified as a carcinogen and consumer perception that carcinogens are dangerous and unacceptable, regardless of what the data show. In fact, there are many chemicals that have been found to cause tumors in rodents at high doses that do not pose a safety concern at low levels more representative of human exposure. Should these be banned? What if a high dose rodent carcinogen actually shows evidence of anti-carcinogenicity that may be more relevant to human exposures, and yet is still classified as a carcinogen on the basis of a rodent bioassay? Literature calling for a re-evaluation of methods used to classify substances as carcinogens has been growing. The importance of this can only be fully understood by investigating the consequences of the current classification system and recognizing that not only does it fail to improve the safety of our food, but in fact contributes to misunderstanding and fear on the part of many consumers who believe that their food is not safe. Other consumers may start to tune-out messages that involve hazard-based labels because those messages have become so pervasive that they are no longer effective. This paper provides a case-study of BHA, an antioxidant that plays an important role in protecting food safety through its antioxidant properties. A robust database and understanding of the MOA has shown that although BHA has been found to be a rodent carcinogen following chronic exposure to relatively high doses, it poses no safety concern to humans at the levels found in our diet. Yet, BHA is found on many lists of banned chemicals or chemicals of concern to the lay public based solely on its hazard classification. Current regulatory requirements and credentialing programs based on hazard can lead to unfounded restrictions and can increase the challenge to communicate safety vs risk to

¹ <https://www.ewg.org/foodscores/ingredients/7519-ButylatedHydroxyanisoBHA/search/>.

² <https://foodbabe.com/ingredients-to-avoid/>.

³ <https://www.stopkillingmykids.com/bht-bha/#:~:text=Processed%20foods%20contain%20a%20variety,health%20and%20are%20completely%20unnecessary.>

⁴ <http://www.safecosmetics.org/get-the-facts/chemicals-of-concern/butylated-compounds/>.

⁵ (https://oregon.public.law/rules/oar_333-016-2020).

⁶ <https://legislation.nysenate.gov/pdf/bills/2019/A9765>.

consumers. It is imperative that we ensure human relevance of the endpoint and the exposure conditions associated with carcinogenicity as being fundamental to hazard identification so that data from traditional animal studies and new alternative methods do not contribute to hazard classification that does not benefit human health.

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CRediT authorship contribution statement

Susan P. Felter: Conceptualization, Funding acquisition, Project administration, Writing - original draft, toxicology literature, Writing - review & editing. **Xiaoling Zhang:** Writing - original draft, toxicology literature, Writing - review & editing. **Chad Thompson:** Data curation, Writing - original draft, toxicology literature, Writing - review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SPF and XZ are employed by Procter & Gamble. CT is employed by ToxStrategies, a consulting company whose clients come from a broad range of industries. We have no financial interests/personal relationships to declare as potential competing interests.

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