September 20, 2011

Members of the Carcinogen Identification Committee

Ms. Cynthia Oshita
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P.O. Box 4010, MS-19B
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Re: Prioritization of Butylated Hydroxytoluene (BHT), in “Prioritization: Chemicals for Consultation by the Carcinogen Identification Committee” [Notice, 7/22/11]

Dear Chairperson Mack, Members of the Carcinogen Identification Committee (CIC), and Ms. Oshita:

On behalf of a BHT Coalition made up of the Grocery Manufacturers Association, the Personal Care Products Council, the Consumer Specialty Products Association and the North American Metal Packaging Alliance in Washington, DC, I am writing to recommend that Butylated Hydroxytoluene (BHT) be given a “No Priority” or “Low Priority” for further carcinogenicity review by the CIC. As described below, the coalition’s member companies use BHT in a large number of various food and consumer products.

The subject notice announced the beginning of a 60-day public comment period on 39 chemicals, including BHT, and these chemicals will be discussed at the October 12-13, 2011 meeting of the CIC. Following this submission, I can provide electronic copies of any references cited herein (those not included in the OEHHA 3-page “Summary Document” for BHT), so they can be made available to CIC members upon request. However, I have included with my submission key selected articles most critical to my evaluation.

By way of my background for evaluating the carcinogenicity of BHT, I served as Chairman of the Toxicology Subgroup of the International Life Sciences Institute-Nutrition Foundation’s (ILSI-NF) Antioxidant Technical Committee from 1984 to 1991. Our Committee was involved in a significant, global scientific effort going back to the early 1980’s in examining the safety of various food antioxidants, including BHT, as well as sponsoring major research studies. The...
Committee sponsored a major symposium on *Food Antioxidants: International Perspectives* April 21-23, 1986 in Washington, DC and the proceedings were subsequently published in a special issue of Food and Chemical Toxicology in late 1986 (see References). This effort helped to confirm BHT’s continued safe use globally in foods as well as the absence of carcinogenicity concern by worldwide regulatory agencies. Our Committee was very actively involved in Proposition 65 listing and risk assessment activities on butylated hydroxyanisole (BHA), a related antioxidant that was listed in 1990 by the Authoritative Bodies listing mechanism.

I have carefully reviewed OEHHA’s 3-page “Summary Document” for BHT as well as the extensive, historical background literature on BHT’s possible carcinogenicity, and I appreciate this opportunity to offer the following comments for your consideration.

**EXECUTIVE SUMMARY**

**Butylated Hydroxytoluene (BHT)** should be given a “No Priority” or a “Low Priority” for further carcinogen listing consideration for the following reasons:

1. **No Authoritative Body has Classified BHT as Causing Cancer:** BHT has not been formally identified as causing cancer by any of Proposition 65’s five Authoritative Bodies, including the U.S. National Toxicology Program (NTP), the International Agency for Research on Cancer (IARC) or the U.S. Food and Drug Administration (FDA). BHT does not meet any of the “Sufficient Evidence” of carcinogenicity criteria required by an Authoritative Body listing.

2. **FDA and EPA Permit Many Regulated and Approved Uses of BHT in Food, Cosmetic, Other Consumer Products and Pesticides:** BHT has been thoroughly evaluated for its carcinogenic potential and has been found safe by both FDA and EPA, two of Proposition 65’s Authoritative Bodies, at current permitted use levels in many products. If FDA had ever determined BHT to be an animal or human carcinogen, the Delaney Clause of the Food Additives Amendment of 1958 would have necessitated its banning for food uses.

3. **BHT has not been Shown to be an Animal Carcinogen in Numerous Lifetime Oral Bioassays:** BHT has been tested up to extremely high doses in numerous chronic oral carcinogenicity bioassays in various species of rats and mice. None of these bioassays, alone or taken together, presents credible scientific evidence that BHT causes cancer in experimental animals. Where hepatocellular tumors have been observed in two bioassays due to BHT treatment, many expert reviewers have dismissed this finding of liver tumorigenicity for reasons noted in my subsequent “Detailed Evaluation” section.

4. **Only One Epidemiological Study is Available for BHT Evaluation:** I disagree with OEHHA that no published studies or data are available in the epidemiologic literature to evaluate BHT’s possible carcinogenicity in humans. One such study, an investigation conducted by the widely recognized Netherlands Cohort Study group, found no significant association with stomach cancer risk for the usual intake of low levels of BHA and BHT.
5. **BHT is Widely Considered to be Non-Genotoxic:** Based upon extensive published reviews of BHT’s genotoxicity database, it is concluded by most experts that the weight of the evidence confirms that BHT is not genotoxic. The overwhelming majority of published BHT genotoxicity studies has reported negative results in both *in vitro* and *in vivo* test systems. In addition, the effect of BHT on the genotoxicity of many well-known genotoxic agents has also been extensively investigated, and it appears that a large majority of about 55 separate endpoints shows BHT in combination with other agents to be decreasing their genotoxic activity.

6. **BHT Can Inhibit and Promote Tumorigenicity when Administered with Known Carcinogens and Mutagens:** BHT has been extensively studied for its ability to moderate the animal tumorigenicity of a very large number of known carcinogens and mutagens. Almost 100 such endpoints have been reported in the literature, and it appears that there is a fairly equal distribution between the inhibitory and promotional effects of BHT. It is important to note, however, that since there is a huge variety of animal species, doses, routes of exposure, timing of BHT administration and other experimental factors, it is difficult to come to a definitive conclusion about inhibition vs. promotion. Because of the reported concern over animal liver tumorigenicity, Dr. Williams’ research group reported a series of studies with well-known liver carcinogens and found that BHT actually reduced the tumorigenic effects of these carcinogens in the liver.

7. **Structure Activity Considerations - BHT vs. BHA:** While both antioxidants do have chemical structure similarities, BHA was classified by IARC as Group 2B, “possibly carcinogenic to humans” based on “sufficient evidence” in experimental animals. BHT, on the other hand, was classified by IARC as Group 3, “not classifiable as to its carcinogenicity to humans” based on “limited evidence” in experimental animals. Furthermore, the *NTP Report on Carcinogens* classified BHA as “reasonably anticipated to be a human carcinogen,” while the same report does not include BHT. Therefore, BHT’s structural similarity to BHA does not adequately predict its carcinogenic effects.

8. **Gary Williams, M.D. has Published a Comprehensive Safety (Hazard) Assessment of BHT (1999) and has Concluded that BHT does not Pose Any Cancer Hazard to Humans:** Dr. Williams and his colleagues were extensively involved in BHT experimental animal and genotoxicity studies during the 1980’s and early 1990’s. Their hazard assessment review paper supports the conclusion that the use of BHT as a food additive does not pose any cancer hazard to humans.

**DETAILED EVALUATION OF BHT**

1. **No Authoritative Body has Classified BHT as Causing Cancer**

BHT has never been formally identified as causing cancer by any of Proposition 65’s five Authoritative Bodies, including the U.S. National Toxicology Program (NTP), the International Agency for Research on Cancer (IARC) or the U.S. Food and Drug Administration (FDA).
OEHHA pointed out in their Prioritization background materials that “Candidate chemicals that are candidates for listing via an administrative listing mechanism were not screened.” I agree with OEHHA that BHT does not qualify as a candidate for an Authoritative Body listing, since it does not meet the criteria of “Sufficient Evidence” of carcinogenicity required by an Authoritative Body listing (27 CCR Section 25306):

a. The NTP reported on a chronic lifetime carcinogenicity bioassay of BHT in 1979, and concluded that “…under the conditions of this bioassay, BHT was not carcinogenic for F344 rats or B6C3F1 mice.” The animals were fed doses of either 3,000 or 6,000 ppm and showed “no evidence” of carcinogenicity in male or female rats or mice.

b. BHT has also never been classified as a carcinogen in the NTP Report on Carcinogens (NTP, 2011).

c. IARC (1986) concluded in their most recent evaluation of BHT, a 1986 Monograph Report, that BHT was classified as Group 3, “not classifiable as to its carcinogenicity to humans…” based on “limited evidence” in experimental animals and the absence of any available data to evaluate the carcinogenicity to humans. IARC’s conclusion did take into consideration the published results of the Olsen et al. (1986) lifetime carcinogenicity study in male and female Wistar rats, cited by OEHHA as evidence of having a carcinogenic effect. IARC dismissed the liver tumors observed in the Olsen study because of large differences in survival between control and treated groups, resulting from BHT’s action in extensively prolonging the lives of the treated animals well past the time of natural deaths of control animals (see discussion in section 3 below).

d. FDA extensively evaluated the carcinogenicity of BHT during the second half of the 1980’s and early 1990’s. The agency concluded that it was not a carcinogen, and has therefore allowed to date its continued, long-approved uses in many food, cosmetic and other consumer products.

2. FDA and EPA Permit Many Regulated and Approved Uses of BHT in Food, Cosmetic, Other Consumer Products and Pesticides

OEHHA noted correctly in their “Summary Document” that “…BHT is used as an antioxidant/preservative in foods at levels ranging from 10 to 200 ppm. Current food regulations establish a maximum content of 0.02 percent for all antioxidants combined. Industrial applications of BHT include use as an antioxidant in rubber, petroleum, and plastic products.” Details about some of these food and consumer product uses are provided here.

By way of background related to BHT’s food uses, in 1950 the Delaney Committee started a congressional investigation of the safety of additives that laid the foundation for the Food Additives Amendment and the Color Additive Amendments (FDA, 2011). Rep. James Delaney, D-NY., later submitted a change to the bill proposing the Food Additives Amendment by inserting the Delaney Clause, which prohibited the approval of any food additive shown to induce cancer in humans or animals in studies with a relevant route of exposure. The resulting 1958 Delaney Clause amendment to the Federal Food, Drug and Cosmetic Act bars FDA from
approving any food additive that causes cancer in man or in animal tests, and the courts have held that the Delaney Clause is not subject to any exception for *de minimis* levels; it is an absolute ban.

FDA has continued to permit various food uses of BHT, which attests to the fact that the scientific evidence does not indicate to FDA that BHT is a carcinogenic hazard. If FDA had made such a carcinogenic ruling for BHT many years ago, the law would have demanded its outright ban from food products. Scientific data that became available in the years since BHT was approved for uses in or on food have not prompted FDA - nor any other expert food safety or regulatory body for that matter - to question or change BHT’s non-carcinogen status.

By way of background related to BHT’s use in cosmetic products, the Cosmetic Ingredient Review (CIR) was established in 1976 as an independent safety review program for cosmetic ingredients. The CIR Expert Panel consists of independent experts in dermatology, toxicology, pharmacology and veterinary medicine, and the CIR includes participation by the FDA and the Consumer Federation of America. As summarized by the CIR Expert Panel in its safety assessment of BHT, the chemical is an important ingredient used in a wide range of cosmetic formulations at low concentrations, ranging from 0.0002% to 0.5% (CIR, 2002). BHT acts as an antioxidant by preventing or slowing the deterioration of cosmetics and personal care products caused by chemical reactions with oxygen. Thus, as with many foodstuffs, BHT is an important ingredient that helps to maintain the quality, integrity and safety of cosmetic products. The CIR Expert Panel concluded from their comprehensive review of animal and human data that “…BHT is safe as used in cosmetic products” (page 81).

BHT is also permitted as an indirect food additive in numerous food contact applications, specifically as an antioxidant preservative for metal packaging adhesives and coatings. In addition, BHT is used as an antioxidant preservative for pesticide products and fragrances. By way of background related to BHT’s antioxidant use as an inert ingredient in pesticide formulations, U.S. EPA has permitted such uses as required under the Food Quality Protection Act (EPA, 2005, 2011). EPA (2011, item 517, page 10 of 76) lists BHT as an approved inert ingredient for nonfood use pesticide products.

The most recent safety evaluation of both BHT and BHA is contained in EPA’s “Inert Ingredient Reassessment - BHA and BHT” document (EPA, 2005). EPA noted that both antioxidants have typical concentrations in food-use pesticide products < 0.2% and that both also have exemptions from the requirement of a tolerance when used as inert ingredients in pesticide formulations when applied to growing crops or raw agricultural commodities after harvest. EPA (2005) came to the following conclusion in its reassessment document for the use of BHT and BHA as inert ingredients in pesticide formulations:

“Taking into consideration all available information on BHA and BHT, including their long history of use as direct food additives and their use in cosmetics, pharmaceuticals, and personal care products, it has been determined that there is a reasonable certainty that no harm to any population subgroup will result from aggregate exposure to BHA or BHT when used as inert ingredients in pesticide formulations when considering dietary
exposure and all other non-occupational sources of pesticide exposure for which there is reliable information.” [page 2 of 20]

3. **BHT has not been Shown to be an Animal Carcinogen in Numerous Lifetime Oral Bioassays** (OEHHA summary information appears in italics):

BHT has been tested up to extremely high doses in numerous chronic oral carcinogenicity bioassays in various species of rats and mice. None of these bioassays, alone or taken together, presents credible scientific evidence that BHT causes cancer in experimental animals. Each of these chronic bioassays will be described in some detail below.

**NCI/NTP (1979).**

- 107 to 108-week diet studies in male and female B6C3F1 mice
  - Increase in alveolar/bronchiolar carcinomas or adenomas (by pairwise comparison) in females
  - No treatment-related increases in males
- 105-week diet studies in male and female Fischer 344 rats
  - No treatment-related increases in males or females

The NCI/NTP (1979) bioassay of BHT in the diet at doses of 3,000 and 6,000 ppm came to the following conclusion (as noted in the report’s Summary, page v), but does not conclude that BHT is a carcinogen in the female mouse lung:

“It is concluded that under the conditions of this bioassay, BHT was **not carcinogenic** for F344 rats or B6C3F1 mice.” [emphasis added]

The Summary goes on to explain the female mouse lung finding:

“Alveolar/bronchiolar carcinomas or adenomas occurred in the female mice at a significant incidence in the low-dose group (P=0.009) but not in the high dose group, and the incidences were not significantly dose related (control 1/20, low-dose 16/46, high-dose 7/50). Thus, these lung tumors in the female **cannot clearly be related to** the administration of the BHT.” [emphasis added]

The Summary also notes, as pointed out correctly by OEHHA, that no tumors occurred in either male or female rats or male mice at incidences that were significantly higher in dosed groups than in the corresponding control groups. As a matter of relevance to NTP as an authoritative body, this bioassay is also officially reported by NTP in its **NTP Technical Report No. 150** (NTP, 1979), since these older NCI bioassays were incorporated into NTP’s current bioassay database. The current **NTP Technical Reports Index** citation for BHT contains the designation of “N” (defined as “Negative” or “No Evidence” of Carcinogenicity) for both male and female rats and male and female mice:

[http://ntp.niehs.nih.gov/?objectid=070510F7-946E-0334-8C3427E3D9734FD0](http://ntp.niehs.nih.gov/?objectid=070510F7-946E-0334-8C3427E3D9734FD0)
Therefore, the key findings of the NCI/NTP 2-year bioassay of BHT in rats and mice do not provide “Sufficient Evidence” of a carcinogenic effect, lending no support for the possible listing of BHT as a carcinogen under Proposition 65.

Hirose et al. (1981).

- 104-week diet studies in male and female Wistar rats
  - No treatment-related findings in males or females

There is general agreement by all who have reviewed this published bioassay that BHT was found not to be carcinogenic in Wistar rats fed BHT for two years. The rats (57 of each sex) were maintained on a diet containing 0.25 % and 1.0 % BHT for 104 weeks, and the control groups were comprised of 36 rats of each sex. The research team, which was led by Dr. N. Ito, Japan’s legendary carcinogenesis expert, reported that histopathological examinations revealed a variety of tumors in treated animals at the end of the study, but their incidence was not significantly different from that in controls and there were no dose-response relationships found. The authors concluded: “This experiment showed no carcinogenic effect of BHT on rats.”

Shirai et al. (1982).

This long-term bioassay study in B6C3F1 mice was not cited by OEHHA, but it was conducted in Dr. Ito’s laboratory in Japan, the same laboratory that conducted the Hirose et al. (1981) Wistar rat study described above. Groups of approximately 50 male and 50 female B6C3F1 mice were given BHT at concentrations of 200, 1,000 or 5,000 ppm in their diet for 96 weeks, followed by a basal diet for 8 weeks and then sacrificed. Similar groups of male and female controls were given basal diet throughout the 104 weeks. Neither survival rates nor food consumption differed between treated and control groups. No significant changes attributable to BHT treatment were found in the hematological examinations or serum and urine analyses. Tumors were found in many organs, especially the lungs, liver, lymph nodes and spleen, in both the treated and control groups. The difference in incidence between the BHT-treated groups and the controls was not statistically significant for any type of tumor, so none were related to BHT treatment. The authors concluded that this study provided no evidence of BHT carcinogenicity in mice. Therefore, this study confirmed the findings of non-carcinogenicity in the NCI/NTP (1979) study also in B6C3F1 mice described above.

Lindenschmidt et al. (1986).

- 10-month diet studies in male and female C3H mice:
  - Increase in spontaneous development of liver tumors (by pairwise comparison) in males
  - No treatment-related increases in females

This study, being of only 10-months duration, does not meet the accepted criteria for a modern-day lifetime carcinogenicity bioassay. The mice were fed a diet containing 0.05% and 0.5 % (500 and 5,000 ppm) BHT, and after 10 months, male but not female animals had a significantly increased incidence of liver tumors compared to animals kept on a BHT-free control diet. It has
been noted in various reviews (including JECFA/WHO, 1996, section 2.2.2.1) that historical control data were not available in comparable 10-month studies in this mouse strain and that the incidence of spontaneously-occurring hepatic tumors in C3H mice is known to be modified by population density, level of dietary protein and caloric intake. The incidence of hepatic tumors in a 12-month study in this strain ranged from 6-13% for females and 41-68% for males (Peraino et al., 1973, also cited in section 2.2.2.1). Thus, JECFA/WHO noted that the reported incidence of hepatocellular tumors was not significantly different from other controls of similar age, leading them to conclude that the results of this short-term study do not provide scientifically reliable evidence for a carcinogenic effect of BHT.

Olsen et al. (1986).

- Two-generation diet studies in male and female Wistar rats dosed for entire lifespan:
  - Increase in hepatocellular adenoma, carcinoma, or adenoma and carcinoma combined (by pairwise comparison and trend) in males
  - Increase in hepatocellular adenomas (by pairwise comparison) in females

Many BHT researchers and scientific reviewers over the past 25 years have focused much attention on the results of this two-generation feeding study of BHT in Wistar rats, the same strain studied by Hirose et al. (1981; described above) with no observed carcinogenic effect, because of its reported finding of BHT carcinogenicity.

Groups of 60, 40, 40 and 60 F0 Wistar rats of each sex were fed a semi-synthetic diet containing BHT in concentrations providing intakes of 0, 25, 100 or 500 mg/kg bw/day, respectively. The F0 rats were mated and groups of 100, 80, 80 or 100 F1 rats of each sex were formed. After weaning, the highest dose (500 mg BHT/kg bw/day) was lowered to 250 mg/kg bw/day for the F1 rats. At weaning, treated F1 rats had lower body weights than the controls, the extent of the reduction being dose related, and this effect, which persisted throughout the study, was most pronounced in the males.

Dose-related increases in the numbers of hepatocellular adenomas and carcinomas were statistically significant (at $P < 0.05$) only in male F1 rats when all groups together were tested for heterogeneity or analysis for trend. However, the increase in hepatocellular tumors in treated female F1 rats was only statistically significant for benign adenomas (at $P < 0.05$) in the analysis for trend. Therefore, as a result of BHT treatment, this study produced a significant increase in combined benign and malignant tumors only in one organ (liver) and in one sex (male). Although tumors were found in other organs of some of the treated rats, their incidence was not significantly different from that in controls. In both sexes, the lowest dose of 25 mg/kg bw/day, had no effect on tumor induction.

It is important to point out that the survival of BHT-treated F1 rats of both sexes was significantly better than that of the controls ($P < 0.001$). This important finding has been discussed by most reviewers of this study, since all the hepatocellular tumors produced by BHT were detected when the F1 rats were more than 2 years old, and most were actually found in animals examined at terminal sacrifice at 141-144 weeks (2 ¾ years of age). In fact, for both benign and malignant hepatocellular tumors, the very first tumor was found after 114 weeks of
treatment in both sexes, a time period 10 weeks or greater beyond the conventional 104-week chronic bioassay. In males, the first carcinoma was seen at 117 weeks in controls, but not until 141 weeks for the mid-dose group and 132 weeks for the high-dose group, so tumor latency was actually increased in BHT-dosed animals compared to controls.

Many scientists have speculated that the liver tumors observed by Olsen et al. (1986) from BHT treatment may have resulted from the longevity-producing effects of the chemical, thus complicating the clear interpretation of the study’s findings. Gary M. Williams, M.D., one of the world’s leading experts on chemical carcinogenicity and animal toxicologic pathology (see also section 8 below), has published many experimental studies and expert reviews on BHT. He and his colleagues pointed out that in the Olsen study, “…the very high mortality in the control animals compromised the statistical comparison to BHT exposure groups” (Williams et al., 1999). Williams’ research group further concluded that the findings in this study have not been confirmed in other studies and may be attributable to study conditions, not to the administration of BHT itself.

Furthermore, Olsen et al. (1986), owing to the difficulties they discussed in interpreting the observed differences in hepatocellular tumors between control and treated animals, concluded that “The role of BHT in the development of hepatocellular tumors requires further elucidation.” Dr. Gunna Wurtzen, the senior author of the Olsen et al. (1986) study, commenting subsequently on BHT safety and her own study, in her review of the shortcomings of toxicity testing strategies for several antioxidants (Wurtzen, 1990), concluded that:

“BHT has not been found to be mutagenic. In a long-term rat study with in utero exposure, BHT showed hepatocarcinogenic effects, but these alterations were only seen at a very late stage of the experiment, which lasted for 144 wk. This exceeds the period set by guideline tests and so effects are likely to occur as a result of ageing of the animals.”

That most of these BHT-related tumors were found at terminal sacrifice (2 ¾ years) stands in contrast to the lack of a similar tumorigenic effect in the 2-year study by Hirose et al. (1981) in the same rat strain.

Inai et al. (1988).

BHT was orally administered at concentrations of 1% and 2% (10,000 and 20,000 ppm) of the diet to B6C3F1 mice for 104 weeks. The two groups of 50 males received calculated average doses of 1,640 and 3,480 mg/kg bw/day and the two groups of 50 females received doses of 1,750 and 4,130 mg/kg bw/day. Treated animals underwent a 16-week recovery period prior to pathological examination. The average body weights of both male and female mice given BHT showed a dose-related reduction compared to controls. For female mice given the high dose of BHT, the incidence of mice with tumors was significantly lower (P < 0.01) and the survival time of mice with tumors was significantly longer (P < 0.01) than that of control mice. For male mice the survival times of mice with tumors were not significantly different between treated and control groups.
The tumor findings due to BHT treatment differed between the males and females. In male mice administered BHT, the incidence of mice with benign hepatocellular adenomas was significantly increased (P < 0.01) only at the high dose, but there was no significant increase in malignant hepatocellular carcinomas (22, 26 and 17% incidences for control, low and high doses, respectively). In addition, the incidences of male mice with other tumors and female mice with any tumors were not significantly increased as a consequence of BHT administration. Therefore, the authors concluded that their study indicated that BHT increased the incidence of benign liver tumors in the B6C3F1 male mouse, although they did point out that the observation period (120 weeks: 104 weeks of treatment plus a 16-week recovery period) was longer than in conventional 104-week carcinogen bioassays. Furthermore, in regards to the association between longer survival times and increased tumor incidence described above, they noted that “…most of the hepatocellular tumors …were thought to have developed at a late stage of the experimental period, because the mice that survived longer showed a higher incidence of hepatocellular tumor.” This observation is in line with the same association found for hepatocellular tumors in the Olsen et al. (1986) Wistar rat study.

**Williams et al. (1990a).**

BHT was fed in the diet to male F344 rats in two chronic feeding studies. The researchers acknowledged that these studies were not designed as definitive chronic bioassays, but concluded that valuable information was obtained from them when taken together with the results in a companion paper on genetic and cellular effects (Williams et al., 1990b: see section 5 below). In one study with 21 animals in each group, feeding BHT for 76 weeks at concentrations of 100, 300, 1,000, 3,000 and 6,000 ppm produced no significant increase in benign or malignant neoplasms at any site. Feeding of BHT at the top two doses significantly reduced the body-weight gains compared to controls. In a second study, feeding of 27 animals with 12,000 ppm BHT for 110 weeks had no significant benign or malignant neoplastic effects at any site. Feeding of BHT at this highest dose also significantly reduced the body-weight gains compared to controls. The incidence of hepatic cell neoplasms was slightly increased in the group fed 6,000 ppm BHT for 76 weeks and was significantly lower in the group fed 12,000 ppm BHT for 110 weeks, but neither was significantly different compared with the control groups.

The authors concluded that “…BHT exerted no carcinogenic effect in rats…” Their two studies also examined the possible BHT enhancement of altered hepatocellular foci, a well-recognized, pre-neoplastic effect that known liver carcinogens and tumor promoters produce as precursor lesions to hepatocellular neoplasms. They noted, however, that BHT did not enhance the appearance of these hepatocellular foci in their studies. The authors did acknowledge that under some conditions [i.e., the BHT bioassays of Olsen et al. (1986) in rats and of Inai et al. (1988) in mice], an increase in hepatocellular neoplasms had been observed, but that “…any effect of BHT on liver carcinogenesis is only apparent at very high doses…” and that “…the observed effects on liver carcinogenesis may also involve cytotoxicity.”

4. **Only One Epidemiological Study is Available for BHT Evaluation**

**Botterweck et al. (2000).**
The association between dietary intake of BHA and BHT and stomach cancer risk was investigated in the Netherlands Cohort Study that started in 1986 among 120,852 men and women aged 55 to 69 years. A semi-quantitative food frequency questionnaire was used to assess food consumption. Information on both BHA or BHT content of cooking fats, oils, mayonnaise and other creamy salad dressings and dried soups was obtained by chemical analysis, a Dutch database of food additives (ALBA) and the Dutch Compendium of Foods and Diet Products. After 6.3 years of follow-up, complete data on BHA and BHT intake of 192 incident stomach cancer cases and 2,035 subcohort members were available for case-cohort analysis. Mean intake of BHA or BHT among subcohort members was 105 and 351 µg/day, respectively. For consumption of mayonnaise and other creamy salad dressings with BHA or BHT, no association with stomach cancer risk was observed. A statistically non-significant decrease in stomach cancer risk was observed with increasing BHA and BHT intake, with a rate ratio of highest/lowest intake of BHA = 0.57 (95% CI, 0.25-1.30) and BHT = 0.74 (95% CI, 0.38-1.43). In this study, no significant association with stomach cancer risk was found for usual intake of low levels of BHA and BHT.

5. BHT is Widely Considered to be Non-Genotoxic

OEHHA cited the most recent comprehensive review of the genotoxicity database on BHT (Bomhard et al., 2002), and in their Summary Document they included mention of 11 types of test results covering both \textit{in vitro} and \textit{in vivo} test systems. With regard to point mutation assays, the vast majority of these genotoxicity tests produced negative results, which is in line with the published literature database and other expert scientific reviews. Bomhard et al. (2002) noted that these tests included \textit{in vitro} studies on various bacterial species and strains and on various types of mammalian cell lines, as well as \textit{in vivo} studies on \textit{Drosophila melanogaster}, silk worms and also the mouse specific locus test (involving long-term exposure). They concluded that “Together these studies convincingly show the absence of a potential for BHT to cause point mutations.” (abstract, page 187).

Bomhard et al. (2002) also cited a great number of studies on many cell types and species to examine the potential of BHT to cause chromosome aberrations. They noted that \textit{in vitro} studies have been published using plant cells and the WI-38, CHL, CHO and V79 mammalian cell lines, and \textit{in vivo} studies have been carried out on somatic and/or germ cells of \textit{Drosophila melanogaster}, rats and mice. The authors concluded (abstract, page 187):

“Nearly all studies, especially those using validated test systems, indicate that BHT lacks clastogenic potential. \textit{In vitro} studies on bacterial, yeast and various mammalian cell lines including DON, CHO, CHL cells and primary hepatocytes demonstrate the absence of interactions with or damage to DNA. \textbf{Taking all the existing data into account, the weight of evidence suggests that BHT does not represent a relevant mutagenic/genotoxic risk to man.}” [emphasis added]

In 1986, an IARC Monograph Working Group came to a similar conclusion on BHT’s genotoxicity based on the earlier BHT database (IARC, 1986; from \textit{Summary of Data Reported and Evaluation}, p. 190):
“Butylated hydroxytoluene did not induce DNA damage in *Bacillus subtilis* or mutation in *Salmonella typhimurium*. It did not induce chromosomal aberrations in plants or mutation or chromosomal aberrations in *Drosophila melanogaster*. In one study, it was reported to be mutagenic to cultured Chinese hamster cells in the presence of an exogenous metabolic system. Binding of butylated hydroxytoluene to the DNA of liver of rats treated *in vivo* has been reported. It did not induce micronuclei in bone marrow or dominant lethal mutations in mice. It induced sperm abnormalities in mice.”

Dr. Gary Williams and his colleagues came to a similar conclusion on BHT’s non-genotoxicity in their detailed study using three *in vitro* test systems (Williams et al., 1990b) as well as in their comprehensive review and safety assessment of BHT (Williams et al., 1999; also see section 8 below). Williams et al. (1990b) found no evidence of BHT genotoxicity in the hepatocyte primary culture/DNA repair test, the Salmonella/microsome mutagenesis test and the adult rat liver epithelial cell/hypoxanthine-guanine phosphoribosyl transferase test. They also reviewed the BHT genotoxicity literature up to that date and concluded (page 796-797):

> “Overall, most data on the genotoxicity of BHT are negative, including the present studies. The few positive findings have limitations and have not been confirmed. More investigations of the target organ (i.e. liver) and of DNA interactions need to be conducted, but the currently available data lead us to conclude that BHT is not DNA-reactive.” [emphasis added]

The CIR Expert Panel Report (CIR, 2002) contains an extensive table summarizing about 75 separate BHT genotoxicity studies (Table 8, pages 56-58), with over 90% of them showing no evidence of a genotoxic effect of BHT. In addition, the CIR (2002) also reviewed studies of the effect of BHT on the genotoxicity of many known genotoxic agents (Table 9, pages 59-61), and it appears that a large majority of about 55 separate endpoints shows BHT in combination with other agents to be decreasing genotoxic activity.

Therefore, the numerous expert reviews and evaluations, taken together, demonstrate that the overwhelming weight of the scientific evidence confirms that BHT has not been found to have any appreciable genotoxic activity in a large database of well-conducted *in vitro* and *in vivo* studies.

6. **BHT Can Inhibit and Promote Tumorigenicity when Administered with Known Carcinogens and Mutagens**

OEHHA’s “Summary Document” for BHT cited four studies showing BHT to be a promoter of carcinogenicity and only one study showing BHT’s inhibition of carcinogenicity. In fact, however, there are many dozens of such studies published in the literature, and these have been reviewed by carcinogenicity experts in the field. OEHHA cited Hirose et al. (1993) as the source document that reviewed three of the five studies cited, but this publication is not a primary review document for these kinds of studies, but instead an experimental study performed in Dr. Ito’s laboratory. Hirose et al. (1993) conducted a multi-organ carcinogenesis model study of four antioxidants as modifiers of the carcinogenic effects of several potent carcinogenic initiators in F344 rats showed. They showed that BHT, in a 36-week feeding study, while it enhanced the
development of only thyroid hyperplasias, it also strongly reduced the incidence and multiplicity of colon adenocarcinomas as well as lowered the incidence and multiplicity of renal cell tumors.

In 1986, an IARC Monograph Working Group reviewed and evaluated BHT’s role in inhibiting and promoting the carcinogenicity of many known carcinogens (IARC, 1986; from Summary of Data Reported and Evaluation, p. 191) and concluded:

“When tested in combination with other chemicals (usually, known mutagens or carcinogens), butylated hydroxytoluene often modified the DNA-damaging, mutagenic and clastogenic activities. In most studies, butylated hydroxytoluene reduced the activity of indirectly-acting mutagens or carcinogens.” [emphasis added]

Dr. Gary Williams and his colleagues, in their comprehensive review and safety assessment of BHT (described in more detail in section 8 below), reviewed several key anticarcinogenicity studies of BHT (Williams et al., 1999). They described numerous studies showing that BHT inhibits the carcinogenicity of a variety of carcinogens in different tissues in mice and rats when given at high concentrations of greater than 3,000 ppm (Wattenberg, 1985; Williams, 1993; Williams and Iatropoulos, 1997). They noted that BHT also inhibits liver and mammary gland carcinogenesis in rats (Ulland et al., 1973) and colon carcinogenesis in rats (Weisburger et al., 1977).

Additionally, with an important focus on the two chronic carcinogenicity bioassays (described above) with apparently increased liver tumors in animals, Williams’ group showed that BHT inhibited the hepatocarcinogenicity of both aflatoxin B1 (AFB1) and 2-acetylaminofluorene (AAF) in rats (Maeura et al., 1984; Williams et al., 1986, 1991). In contrast to these high dose studies, Williams et al. (1986) have also shown that BHT administered to rats at 1,000 ppm, starting one week before AFB1 administration and continuing for one week after cessation, decreased liver neoplasia. Also, in a subsequent study in their laboratory, BHT at 125 ppm inhibited the initiation of hepatocarcinogenesis by AFB1 in rats studied over 42 weeks (Williams and Iatropoulos, 1996), and in another study BHT at 100 ppm, fed together with AAF at a low concentration of 50 ppm, inhibited the induction of liver altered foci and reduced the incidence of liver carcinomas by week 76 (Williams et al., 1991).

The CIR Expert Panel Report (CIR, 2002) contains an extensive table of BHT inhibition/promotion experimental results (Table 11, pages 64-70), and of approximately 100 separate endpoints tabulated, it appears that there is a fairly equal distribution between the inhibitory and promotional effects of BHT. It is important to note, however, that since there is a huge variety of animal species, doses, routes of exposure, timing of the administration of BHT and other experimental factors, it is difficult to come to a definitive conclusion about inhibition vs. promotion.

7. **Structure Activity Considerations - BHT vs. BHA**

OEHHA pointed out that BHT is structurally similar to butylated hydroxyanisole (BHA), a listed Proposition 65 carcinogen and IARC Group 2B carcinogen. While I agree that the two compounds are structurally similar phenolics, IARC came to a different conclusion on BHT’s
level of carcinogenicity. BHA was classified by IARC as Group 2B, “possibly carcinogenic to humans,” based on “sufficient evidence” in experimental animals (due to the increased incidence of only forestomach tumors resulting from very high animal doses) and the absence of any epidemiologic data to evaluate the carcinogenicity to humans. Although BHA was added to the Proposition 65 list in 1990 based on this finding of animal forestomach tumors only, it nonetheless has the highest “No Significant Risk Level” (a “Safe Harbor Level” of 4,000 µg/day) adopted in regulation for any listed carcinogen. BHA has continued its long history of safe use in foods and other consumer products across the globe.

BHT, on the other hand, was classified by IARC as Group 3, “not classifiable as to its carcinogenicity to humans” based on “limited evidence” in experimental animals and the absence of any epidemiologic data to evaluate the carcinogenicity to humans. Both of these classifications were determined by the same scientists at the same IARC Monograph Working Group meeting (IARC, 1986).

Furthermore, the NTP Report on Carcinogens classified BHA as “reasonably anticipated to be a human carcinogen,” also based on “sufficient evidence” in experimental animals, while the same report does not include BHT to date (NTP, 2011). Therefore, these two compounds’ structural similarities do not adequately predict their individual carcinogenic effects. In addition, while structure-activity relationships (SAR) and their extrapolations can be valuable when leveraging from a data-rich molecule to a data-poor molecule, they are not appropriate when sufficient data exist for the specific molecule under evaluation.

8. Gary Williams, M.D. has Published a Comprehensive Safety (Hazard) Assessment of BHT (1999) and Concluded that BHT does not Pose Any Cancer Hazard to Humans

Gary Williams, M.D.’s laboratory, as noted above, has been deeply involved in genotoxicity, mechanistic and carcinogenicity studies of BHT since the early 1980’s. In 1999, after almost 20 years involvement with BHT, he and his colleagues published a critical review and safety assessment of BHT as a food antioxidant (Williams et al., 1999). As part of their safety assessment, they reviewed all the significant studies and came to the following conclusions on various BHT endpoints:

**BHT genotoxicity studies** (see studies in Table 3, page 1032, in this reference):

“The weight of evidence, therefore, supports the conclusion that BHT is not genotoxic.”

**BHT carcinogenicity studies** - a review of oral (diet) chronic bioassays of BHT (see studies in Table 4, page 1033, in this reference):

“Overall, these data do not provide convincing evidence that BHT has carcinogenic activity in either mice or rats. Interestingly, 2,2′-methylenebis (4-methyl-6-tert-butylphenol), an antioxidant which is essentially two molecules of BHT and has all attributes of BHT, was also non-carcinogenic at up to 0.1% for 18 months to Wistar rats (Takagi et al., 1994).”
**Studies on BHT mode of action** (see page 1034 in this reference):

“Thus, BHT at high doses can exert tumor-promoting effects, apparently due to blocking of cellular communication channels, but this does not seem to be sufficient for definite enhancement of tumor development when administered on its own.”

**Anticarcinogenicity studies of BHT** (see page 1034 in this reference):

“Thus, the effective chemoprotective concentrations of BHT extend below 1000 ppm to 100 ppm (Williams and Iatropoulos, 1997). Activity at such low concentrations has been suggested to be due to free radical trapping activity (Williams and Iatropoulos, 1997).”

**Overall Conclusions** (see pages 1034-1035):

“Based on the entire body of evidence and data from mechanistic studies, BHT is not genotoxic or reproducibly carcinogenic, although at high doses, 250 mg/kg/day or greater, it was associated with some unconfirmed increases in spontaneous neoplasms and...has some tumor-promoting activity. The overall evaluation of IARC was that BHT is not classifiable as to its carcinogenicity to humans (IARC, 1987). Based on these considerations, we support the conclusion of authorities that the use of BHT as a food additive does not pose any cancer hazard to humans (JECFA, 1996).” [emphasis added]

**CONCLUSION**

BHT has a long record of safe use in many food, cosmetic, consumer and industrial products. The foregoing review and evaluation bears on cancer hazard assessment of BHT based almost exclusively on animal studies. I conclude from my review that BHT poses no carcinogenicity hazard. For a chemical to be listed by Proposition 65, there must be “...a finding by the “state’s qualified experts” that the chemical “has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity” (Health and Safety Code section 25249.8(b)).” With this requirement in mind, I believe that the weight of the evidence for BHT fails to meet the “clearly shown to cause cancer” listing standard in the statute. Consequently, I believe that the overall weight of the evidence available in the literature on BHT’s carcinogenic potential will make it very difficult for the CIC to conclude that BHT has been “clearly shown to cause cancer.”

In conclusion, on behalf of the BHT Coalition, I urge the CIC to give BHT a “No Priority” or “Low Priority” for further listing consideration. If you have any questions, please don’t hesitate to contact me. Thank you for this opportunity to submit these comments on behalf of the coalition.
Sincerely yours,

[Signature]

James R. Coughlin, Ph.D.

On Behalf of the BHT Coalition:
GMA, PCPC, CSPA, NAMPA

BHT Coalition

Grocery Manufacturers Association (GMA).

Based in Washington, D.C., the Grocery Manufacturers Association is the voice of more than 300 leading food, beverage and consumer product companies that sustain and enhance the quality of life for hundreds of millions of people in the United States and around the globe. Founded in 1908, GMA is an active, vocal advocate for its member companies and a trusted source of information about the industry and the products consumers rely on and enjoy every day. The association and its member companies are committed to meeting the needs of consumers through product innovation, responsible business practices and effective public policy solutions developed through a genuine partnership with policymakers and other stakeholders. In keeping with its founding principles, GMA helps its members produce safe products through a strong and ongoing commitment to scientific research, testing and evaluation and to providing consumers with the products, tools and information they need to achieve a healthy diet and an active lifestyle. The food, beverage and consumer packaged goods industry in the United States generates sales of $2.1 trillion annually, employs 14 million workers and contributes $1 trillion in added value to the economy every year.

Personal Care Products Council (PCPC).

Based in Washington, D.C., the Personal Care Products Council (formerly the Cosmetics, Toiletry and Fragrance Association (CTFA)) is the trade association representing the cosmetic and personal care products industry in the United States and globally. Founded in 1894, CTFA has a membership of nearly 600 companies including manufacturers, distributors, and suppliers for the vast majority of finished personal care products marketed in the United States.

Consumer Specialty Products Association (CSPA).

The Consumer Specialty Products Association (CSPA) is a voluntary, non-profit national trade association representing more than 240 companies engaged in the manufacture, formulation, distribution, and sale of consumer specialty products for household, institutional, commercial and industrial use. CSPA member companies’ wide range of products includes home, lawn and garden pesticides, antimicrobial products, air care products, industrial and automotive specialty products, detergents and cleaning products, polishes and floor maintenance products, and various types of aerosol products. These products are formulated and packaged in many forms and are generally marketed nationally. CSPA and its member companies are committed to the safe manufacture, distribution, use and disposal of consumer products, and have instituted a product stewardship program, Product Care®, to promote the production and distribution of safe and effective formulated products that provide desirable benefits for household, commercial, institutional and industrial customers and consumers, and their families, pets and their environment.
North American Metal Packaging Alliance (NAMPA).

The North American Metal Packaging Alliance (NAMPA) is a not-for-profit corporation committed to protecting health through the safety of metal packaging and metal packaged foods. NAMPA’s membership includes companies and associations representing various sectors along the supply chain for the food and beverage packaging industry.

REFERENCES


Other Comprehensive Reviews not specifically cited:


