State of California

Department of Health Services

Memorandum

To : Steven A. Book, Ph.D., Chief
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Date : December 19, 1990

Subject: Risk Specific Intake Level for BHA

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From: Reproductive and Cancer Hazard Assessment Section
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8/571-2084

This memorandum is written to recommend an intake level for butylated hydroxyanisole (BHA) for the purposes of 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; California Health and Safety Code 25249.5 et seq.). The International Agency for Research on Cancer has concluded that the evidence for carcinogenicity of BHA in animals is "sufficient" (IARC, 1987), and classified it as a Group 2B carcinogen ("possibly carcinogenic" to humans). No epidemiological studies on BHA are available (IARC, 1987).

The biochemistry, metabolism and toxicology of BHA have recently been extensively reviewed (IARC, 1986; Ito and Hirose, 1989; Clayson *et al.*, 1990), and these reviews are attached for your reference. Detailed technical information on BHA is available in these reviews and is not repeated in this memorandum.

With regard to its carcinogenicity, BHA has been reported to induce forestomach carcinomas in rats, mice and hamsters (for review, see Ito and Hirose, 1989; Clayson *et al.*, 1990). In rodents, the only mammalian group in which BHA has been adequately tested for carcinogenicity, malignant tumors are observed to derive only from forestomach tissue. Nonmalignant neoplastic and preneoplastic changes, such as adenomatous lesions and hyperplasia, however, were also observed in the lungs of Japanese house shrews (which lack a forestomach) and mice. Enhanced cell proliferation or hyperkeratosis were reported in the lower esophagus in animals lacking a forestomach, including dogs, pigs and monkeys (for review, see Ito and Hirose, 1989). With the exception of the Japanese house shrew, the only studies available in mammalian species lacking a forestomach are of short duration and are not appropriate for determining cancer risk of lifetime exposures.

Based on the negative results of a battery of standardized routine genotoxicity tests (IARC, 1986), BHA is not considered to be genotoxic. The mechanism of BHA carcinogenesis thus is referred to by many researchers as epigenetic, and is postulated to be mediated by enhanced cell proliferation at the forestomach, the site of initial contact (Williams, 1986; Ito *et al.*, 1989). Cell proliferation induced by toxicity has recently been proposed to be a major epigenetic mechanism of carcinogenesis (Ames and Gold, 1990; Cohen and Ellwein, 1990). This is controversial among cancer research scientists because experimental evidence for its proof is lacking at the present time (Marx, 1990).

To assess cancer risk from chronic exposure to BHA, 14 long-term animal cancer bioassays conducted on BHA in rats, mice, hamsters and shrews have been evaluated. The results of these studies are summarized in Table 1. The doses used in these studies have been converted when necessary to units mg/kg-day, based on the assumption that the amount of daily food intake for rats is about 5% of their bodyweight, and for mice and hamsters, 10% of their bodyweight.

All rodent studies examined showed a dose-dependent increase of BHA-induced carcinomas in the forestomach. The Ito *et al.* (1986a) study in Fisher 344 rat is the most appropriate of these for BHA risk assessment. While this study is not the most sensitive study for BHA carcinogenicity, this study contains the best information on dose response at lower dose levels. This lifetime study uses six dose groups, with low doses which are the lowest for all 14 studies. Treatments of 0, 0.125, 0.25, 0.5, 1 and 2% BHA in the diet were used. Ito *et al.* (1986a) estimated these to be equivalent to 0, 54.8, 109.6, 230.4, 427.6 and 1322.6 mg/kg-day. The results of this experiment are shown in Table 1. An increase of forestomach squamous cell carcinomas at the highest dose was observed with a tumor incidence of 11/50 (control 0/50). Nonmalignant papillomas in the forestomach were observed at this dose and the next lower dose of 427.6 mg/kg-day with an incidences of 50/50 and 10/50, respectively (control 0/50). Hyperplasia in the forestomach was observed at all dose levels studied with the lowest dose producing a statistically significant increase in hyperplasia at 109.6 mg/kg-day with an incidence of 7/50 (control 0/50). For the nonmalignant endpoints, the no observed effect levels (NOEL) for forestomach papillomas and for forestomach hyperplasia are 230 mg/kg-day and 54.8 mg/kg-day, respectively.

In addition to forestomach lesions, BHA was also reported to induce increases of lung adenomas in mice (Maru and Bhide, 1982; Chung *et al.*, 1986), and lung adenomatous hyperplasia in Japanese house musk shrews (Amo *et al.*, 1990) in long term studies. In a mouse skin initiation-promotion study, BHA was reported to induce skin papillomas after promotion with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (Sato *et al.*, 1987). The occurrence of benign neoplastic and preneoplastic lesions in the lung and skin indicates that the tumorigenic effect of BHA may not always be confined to the rodent forestomach.

With regard to short-term studies, a total of 17 studies in rats, mice, hamsters, monkeys, dogs and pigs were evaluated. The results of these studies are summarized in Table 2 and Table 3. Of studies on rodents, 9 studies in rats and hamsters reported enhanced cell proliferation or hyperplasia, or both in the forestomach, with a few also reporting forestomach carcinomas.

Studies on animals lacking a forestomach, although short-term, showed induction of cell proliferation and parakeratosis. The results are summarized in Table 3. An increase of cell proliferation was observed in the distal esophagus of cynomolgus monkeys (Iverson *et al.*, 1986), and, in addition to cell proliferation, parakeratosis was also reported in the esophagus of pigs (Wurtzen and Olson, 1986). These findings further suggest that the proliferative effect of BHA can extend to tissues other than the rodent forestomach.

The carcinogenesis of BHA may be mediated by an epigenetic mechanism. If cell proliferation is indeed the primary cause of tumor, the linearized multistage polynomial typically used for cancer risk assessment (DHS, 1985; EPA, 1986, see Appendix A) may not be suitable for estimating the cancer potency of BHA. Two alternatives for risk assessment are 1) the use of a model for epigenetic carcinogenesis, and 2) to estimate allowable doses using an "uncertainty factor". Mathematical models have been proposed accommodate certain characteristics of epigenetic mechanisms (Moolgavkar and Venzon, 1979; Moolgavkar and Knudson, 1981; Bogen, 1989; Bogen, 1990). The validity of these models, however, depends heavily on the suitability of the model for the application and accuracy of the values assigned to model parameters. Experimental data for accurate estimation of most these parameters are not available. Because of the inherent uncertainties associated with model structure and parameter selection, these models may not produce more reliable estimates than the current risk assessment methodology. Thus, the use of the "uncertainty factor" method appears to be a preferable alternative for the determination of an allowable intake level for BHA.

An appropriate marker for cell proliferation in a life-time animal study is hyperplasia. Hyperplasia, thus, is used as the toxicity endpoint for risk assessment. Using the Ito *et al.* (1986a) F344 rat study, the NOEL for forestomach hyperplasia is identified as 54.8 mg/kg-day. By applying an uncertainty factor of 1000, DHS calculates an intake level of BHA of 54.8 μg/kg-day, or 3.8 mg/day for a 70 kg human adult. The uncertainty factor of 1000 includes a factor of 10 to account for interspecies variability, a factor of 10 for intraspecies variation, and an additional factor of 10 for the carcinogenicity.

Alternately, limited evidence exists to consider BHA to be an initiator or a genotoxic agent, such as the induction of nonmalignant papillomas in a mouse skin painting study (Sato *et al.*, 1987) and two positive chromosome aberration studies (Phillips *et al.*, 1989; Matsuoka *et al.*, 1990). In this case, the linearized multistage model and other default risk assessment procedures

(Appendix A) would apply. Using the dose-response data on the induction of forestomach lesions in the Ito *et al.* (1986a) rat study, the animal cancer potencies (q_{animal}) of 1) the combined papillomas and carcinomas, and 2) the carcinomas alone were calculated to be 7.2×10^{-5} $(mg/kg\text{-}day)^{-1}$ and 3.3×10^{-5} $(mg/kg\text{-}day)^{-1}$, respectively (Appendix A, eq. 1). By applying a surface area correction for interspecies scaling, the human cancer potency (q_{human}) was calculated to be 4.4×10^{-4} $(mg/kg\text{-}day)^{-1}$ and 2×10^{-4} $(mg/kg\text{-}day)^{-1}$ (Appendix A, Eq. 4). Accordingly, the doses of BHA associated with a lifetime cancer risk of 10^{-5} for a 70 kg adult are calculated to be 1.6 mg/day and 3.5 mg/day (Appendix A, eq. 6).

The intake level of BHA calculated from the Ito *et al.* (1986a) study by the uncertainty factor method is 3.8 mg/day. The intake level associated with a 10^{-5} cancer risk calculated by the linearized multistage polynomial model is 1.6 to 3.5 mg/day. Thus, a BHA intake of 4 mg/day appears to be a reasonable one for the level posing no significant risk of cancer for the purpose of Proposition 65.

Lauren Zeise, Ph.D.
Acting Chief

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APPENDIX A: METHODOLOGY USED TO DERIVE INTAKE LEVELS POSING SIGNIFICANT RISK

A.1 Cancer Potency as Derived from Animal Data

"Multistage" polynomial

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily intake (d) is often assumed to be (DHS, 1985; EPA, 1986; Anderson *et al.*, 1983):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \cdots + q_j d^j)] \qquad (1)$$

with constraints

$$q_i \geq 0 \text{ for all } i.$$

The q_i are parameters of the model which are taken to be constants and are estimated from the data. The parameter q_0 represents the background lifetime incidence of the tumor. q_1, or some upper bound, is often called the cancer potency, since for small doses it is the ratio of excess lifetime cancer risk to the average daily dose received. For the present discussion, cancer potency will be defined as q_1^*, the upper 95% confidence bound on q_1 (DHS, 1985), estimated by maximum likelihood techniques. When dose is expressed in units mg/kg-d, the parameters q_1 and q_1^* are given in units $(\text{mg/kg-d})^{-1}$. Details of the estimation procedure are given in Crump (1981) and Crump, Guess, and Deal (1977). To estimate potency in animals (q_{animal}) from experiments of duration T_e, rather than the natural lifespan of the animals (T), it is assumed that cancer incidence increases with the third power of age:

$$q_{animal} = q_1^* \cdot (T/T_e)^3 \qquad (2)$$

Following Gold *et al.* (1984) and EPA (Anderson *et al.*, 1983), the natural lifespan of mice and rats is assumed to be 2 years, so that for experiments lasting T_e weeks in these rodents

$$q_{animal} = q_1^* \cdot (104/T_e)^3 \qquad (3)$$

To estimate risk at low doses, potency is multiplied by average daily dose. The risk estimate obtained is referred to by EPA (Anderson *et al.*, 1983) as "extra risk", and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.

Calculation of the lifetime average dose

A.2 Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. As described in the California risk assessment guidelines (DHS, 1985), a dose in units of milligram per unit surface area is assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. Under this assumption, scaling to the estimated human potency (q_{human}) can be achieved by multiplying the animal potency (q_{animal}) by the ratio of human (bw_h) to animal body weights (bw_a) raised to the one-third power when animal potency is expressed in units $(mg/kg\text{-}day)^{-1}$:

$$q_{human} = q_{animal} \cdot (bw_h / bw_a)^{1/3} \qquad (4)$$

A.3 Risk-Specific Intake Level Calculation

The intake level (I, in mg/day) associated with a cancer risk R, from exposure to compound x is

$$I = \frac{R \cdot BW}{q_{human}} \qquad (5)$$

where BW is the body weight, and q_{human} the theoretical cancer potency estimate for humans.

Lifetime cancer risks above 10^{-5} are associated with significant risks of cancer under Proposition 65 (Title 22 California Code of Regulations, Section 12703). Thus for a 70 kg person, the intake levels posing significant cancer risk under Proposition 65 are given by

$$I = \frac{10^{-5} \cdot 70kg}{q_{human}}$$

or

$$I = \frac{0.0007}{q_{human}} \qquad (6)$$

APPENDIX A REFERENCES

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TABLE 1: LONG-TERM BIOASSAYS (FEED STUDIES)

1. Studies in Rats

Strain, Sex (Duration)	Site(s), Histopathology	Doses (mg/kg-day) Incidences						Reference
		0	54.8	109.6	230.4	427.6	1322.6	Ito et al., 1986a
F344, M	Forestomach							JNCI 77(6):1261-65
(104 wks)	hyperplasia	0/50	1/50	7/50	16/50	44/50	50/50	
	papillomas	0/50	0/50	0/50	0/50	10/50	50/50	
	squamous cell carcinomas	0/50	0/50	0/50	0/50	0/50	11/50	
		0				500	1000	Ito et al., 1986b
F344, M	Forestomach							Toxicol Path 14(3):
(104 wks)	hyperplasia	0/23				24/25	26/26	315-323
	papillomas	0/23				21/25	26/26	
	squamous cell carcinomas	0/23				0/25	9/26	
		0		98		414		Ito et al., 1983
F344, M	Forestomach							JNCI 70:343-352
(104 wks)	hyperplasia	0/51		13/50		52/52		
	papillomas	0/51		1/50		52/52		
	squamous cell carcinomas	0/51		0/50		18/52		
		0		108		474		Ito et al., 1983
F344, F	Forestomach							JNCI 70:343-352
(104 wks)	hyperplasia	0/51		10/51		50/51		
	papillomas	0/51		1/51		49/51		
	squamous cell carcinomas	0/51		0/51		15/51		
		0				500	1000	Masui et al., 1986
F344, M	Forestomach							Gann 77:1083-90
(104 wks)	hyperplasia	1/92				92/94	93/94	
	papillomas	0/92				71/94	86/94	
	squamous cell carcinomas	0/92				0/94	13/94	

		0	1000	Nera et al., 1988
F344, M	Forestomach			Toxicol 53:251-68
(104 wks)	hyperplasia	0/40	37/37	
	papillomas	0/40	37/37	
	squamous cell carcinomas	0/40	2/37	
		0	1000	Masui et al., 1987
F344, M	Forestomach			Cancer Res 47:5171-74
(96 wks)	hyperplasia	0/18	18/18	
	papillomas	0/18	18/18	
	squamous cell carcinomas	0/18	3/18	

2. Studies in Hamsters

Strain, Sex (Duration)	Site(s), Histopathology	Doses (mg/kg-day) Incidences			Reference
		0	1000	2000	Masui et al., 1986
Syrian	Forestomach				Gann 77:1083-1090
golden, M	hyperplasia	9/52	53/55	40/40	
(104 wks)	papillomas	0/52	54/55	38/40	
	squamous cell carcinomas	0/52	4/55	4/40	
		0	1000	2000	Ito et al., 1986b
Syrian	Forestomach				Toxicol Path 14(3):
golden, M	hyperplasia	5/12	11/13	3/4	315-323
(104 wks)	papillomas	2/12	12/13	4/4	
	squamous cell carcinomas	0/12	1/13	0/4	

3. Studies in Mice

Strain, Sex (Duration)	Site(s), Histopathology	Doses (mg/kg-day) Incidences			Reference
		0	500	1000	Masui et al., 1986
B6C3F1, M	Forestomach				Gann 77:1083-1090
(104 wks)	hyperplasia	0/39	10/37	35/43	
	papillomas	0/39	5/37	5/43	
	squamous cell carcinomas	0/39	1/37	2/43	
		0	500	1000	Ito et al., 1986b
B6C3F1, M	Forestomach				Toxicol Path 14(3):
(104 wks)	hyperplasia	0/16	8/21	21/22	315-323
	papillomas	0/16	0/21	1/22	
	squamous cell carcinomas	0/16	0/21	0/22	
Swiss, M/F	Lung	0	500		Maru and Bhide, 1982
(15 or 25 mos.)	tumors (not otherwise	1/47	3/22		Cancer Lett 17:75-80
(Unclear reporting)	specified)				

4. Studies in Musk Shrew

Strain, Sex (Duration)	Site(s), Histopathology	Doses (mg/kg-day) Incidences			Reference
		0	520	1040	Amo et al., 1990
Japanese	Lung				Carcinogenesis
house, M	hyperplasia	0/35	15/24	12/18	11(1):151-4
(85 wks)	(adenomatous)				
	adenomas	0/35	1/24	1/18	
		0	810	1560	Amo et al., 1990
Japanese	Lung				Carcinogenesis
house, F	hyperplasia	1/29	14/28	12/22	11(1):151-4
(85 wks)	(adenomatous)				
	adenomas	0/29	1/28	0/22	

TABLE 2: SHORT-TERM STUDIES (RODENT)

1. Studies in Rats

Strain, Sex (Duration, route)	Site(s), Description	Doses (mg/kg-d) Incidences						Comments	Reference
F344, M	Forestomach, prefundic region	0	50	125	250	500	1000		Clayson et al., 1986
(9 days, diet)	thymidine labelling index (%)	2.30	2.46	2.62	4.74	8.10	10.60		Fd Chem Tox 24(10/11):
	ratio to control		1.07	1.14	2.07	3.53	4.62		1171-1182
F344, M	Forestomach	0					1000		
(21 weeks, diet)	thymidine labelling index (%)	5.0					18.4		Tatematsu et al., 1986 Cancer Lett 33:119-124
F344, M	Forestomach	0					1000	Downward hyperplasia was not reversible after termination of BHA treatment.	Masui et al., 1986 Gann 77:854-857
(Dosed	upward hyperplasia								
24 weeks, diet)	observed at 24 wks	NA					10/10		
	observed at 96 wks	0/18					0/18		
	downward hyperplasia								
	observed at 24 wks	NA					10/10		
	observed at 96 wks	0/18					18/18		
	upward papillomas								
	observed at 24 weeks	NA					10/10		
	observed at 96 weeks	0/18					0/18		
	downward papillomas								
	observed at 24 weeks	NA					10/10		
	observed at 96 weeks	0/18					3/18		
F344, M	Forestomach	0				500	1000	Reported on effects in different regions.	Hirose et al., 1987 Carcinogenesis 8(11):
(52 weeks, diet)	hyperplasia	0/10				15/15	15/15		1731-1735
Wistar, M	Forestomach	0	62.5		250		1000	Also studied location and severity of lesions and recovery. Hyperplasia persisted after 4 wk recovery period.	Altmann et al., 1986 Fd Chem Tox 24(10/11):
(90 days, diet)	hyperplasia	0/15	7/10		10/10		10/10		1183-1188
Wistar, F	Forestomach	0	62.5		250		1000		
(90 days, diet)	hyperplasia	0/15	2/10		7/10		10/10		

Charles River Sprague Dawley, M (duration unclear, diet and gavage)	Forestomach	0	500		Newberne et al., 1986 Fd Chem Tox 24(10/11): 1111-1119.
	hyperplasia				
	diet	1/19	16/20		
	gavage	4/20 (vehicle control)	1/18		
	papillomas				
	diet	0/19	2/20		
	gavage	0/20 (vehicle control)	5/18		
	carcinomas				
	diet	0/19	2/20		
	gavage	0/20 (vehicle control)	12/18		

2. Studies in Hamsters

Strain, Sex (Duration, route)	Site(s), Description	Doses (mg/kg-d) Incidences			Comments	Reference
Syrian golden, sex unreported (35 weeks, diet)	Forestomach	0		1000		Moore et al., 1987 JNCI 78(2):289-293
	papillary hyperplasia	0/18		12/12		
	papillomas	0/18		10/12		
	carcinomas	0/18		4/12		
Misaki Syrian golden, M (21 weeks, diet)	Forestomach	0	880	1850	Also studied LVG hamsters -- less sensitive.	Lam L, 1988 Carcinogenesis 9(9): 1611-1616
	hyperplasia	1/10	9/11	9/10		
	papillomas	0/10	7/11	8/10		
Syrian golden, M (20 weeks, diet)	Forestomach	0		1000		Hirose et al., 1986 Carcinogenesis 7(8): 1285-1289
	hyperplasia (severe)	0/15		15/15		
	papillomatous lesions	0/15		9/15		
	thymidine labelling index (%)	12.5		33.9		

3. Studies in Mice

Strain, Sex (Duration, route)	Site(s), Description	Doses (mg/kg-day) Incidences		Comments	Reference
		0	500		
A/J, F (Treated 11 wks, sacrificed at 31 weeks; diet)	Lung adenomas	5/40	11/38	Studied inhibition by BHA - not significant.	Chung et al., 1986 Cancer Res 46:165-168

4. Studies in Mice (Initiation-Promotion)

Strain, Sex (Duration; route)	Site(s), Description	Doses (mg BHA per application) Incidences		Comments	Reference
		0	10 mg (2X per week)		
CD-1, F (dosed 5 wks with BHA or DMSO [control], dosed 47 wks with TPA; skin painting)	Skin papillomas	0/20	4/20	Treatment with DMSO or BHA alone produced no skin papillomas.	Sato et al., 1987 Cancer Lett 38: 49-56

TABLE 3: SHORT-TERM STUDIES (NON-RODENT)

1. Studies in Monkeys

Strain, Sex (Duration, route)	Site(s), Description	Doses (mg/kg-day) Incidences	Comments	Reference
Rhesus, M/F (4 weeks, gavage)	Liver; proliferation of smooth endoplasmic reticulum in high dose group. Fragmentation of nucleolus in 15% of hepatic cells in high dose group.	0, 50, 500	Liver biopsy only.	Allen and Engbloom, 1972 Fd Cosmet Tox 10:769-779
Cynomolgus, F (12 weeks, gavage)	Distal esophagus Mitotic index (%)	0 89.3 357 0.87 0.77 1.66	Extent of histopath uncertain.	Iverson et al., 1986 Fd Chem Tox 24(10/11): 1197-1200

2. Studies in Pigs

Strain, Sex (Duration, route)	Site(s), Description	Doses (mg/kg-day) Incidences	Comments	Reference
Danish Landrace, F (day 1 to day 110 gestation, diet)	Esophagus; proliferative and parakeratotic changes in "a few pigs" in the mid and high dose groups.	0, 50, 200, 400 (No incidence data given)	Original study not intended to examine esophageal effects, thus esophagus was not systematically preserved. Proliferative changes in pig stomach (not esophagus) are common.	Wurtzen and Olsen, 1986 Fd Chem Tox 24(10/11): 1229-1233

3. Studies in Dogs

Strain, Sex (Duration, route)	Site(s), Description	Doses (mg/kg-d) Incidences	Comments	Reference
Beagle, M/F (6 months, diet)	Liver; increased weight, proliferation of the smooth endoplasmic reticulum.	0, 250	Extent of histopath uncertain; emphasis on stomach.	Ikeda et al., 1986 Fd Chem Tox 24(10/11): 1201-1221
Beagle, M/F (6 months, diet)	No hyperplastic changes in esophagus, stomach or duodenum. No effect on mitotic index in esophagus.	0, 60, 110, 220		Tobe et al., 1986 Fd Chem Tox 24(10/11): 1223-1228