MICHIGAN DEPARTMENT OF NATURAL RESOURCES

INTEROFFICE COMMUNICATION

August 25, 1994

TO: File for Butylated hydroxytoluene (BHT) CAS #128-37-0

FROM: Mary Lee Hultin, Toxics Unit

SUBJECT: Screening level for Butylated hydroxytoluene (BHT) CAS #128-37-0

The American Conference of Governmental Industrial Hygienists (ACGIH, 1993) has set an eight hour TWA TLV for BHT of 10 mg/m^3. This limit is identical to the occupational exposure limits set by OSHA [PEL, 8 hr. TWA] and NIOSH [REL, 10 hr. TWA] (NIOSH, 1990). The basis for this limit is not clear, however it does not appear to be based on carcinogenicity data. The carcinogenicity of BHT has been studied extensively, but results are difficult to interpret. IARC (1986;1987) has classified BHT into Group 3 with respect to carcinogenicity, having no adequate human data and limited evidence in animals.

There are both positive and negative bioassays on carcinogenicity in a variety of species. There is evidence that BHT acts as a promoter, initiator, inhibitor, complete carcinogen and none of the above. Since BHT is a commonly used antioxidant in food products and packaging, most studies have been conducted via oral administration. No studies using the inhalation route of administration were located on the carcinogenicity of BHT.

The largest number of positive bioassays involve hepatocarcinogenic effects:

> Olsen, et al (1986) found liver adenomas and carcinomas in F1 Wistar rats in a two generation study. Dietary doses were 1, 25, 100, 250 and 500 mg/kg. Complicating the interpretation of this study are the facts that survival in the control animals was significantly lower than in treated rats (possibly due to the antioxidant properties of BHT); and that tumors did not develop until the F1 rats were greater than 2 years old.
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> Clapp, et al. (1973a) discovered the development of hepatic bile duct hyperplasia in male BALB\c mice dosed for 10 months; however, this finding was not repeatable by the same authors using a larger group of mice (though BHT in the second study potentiated hepatic cyst formation and lung tumor development in diethylnitrosamine-initiated mice). Doses in both studies were 0.75% in diet.

Inai, et al. (1988) found increased incidence of hepatocellular adenomas in male B6C3F1 mice in a 2 year study. Doses were 0, 1% and 2% of the diet. Like the Olsen data, survival was decreased in the controls. However, unlike Olsen's findings, the appearance of the first tumor was at 62 weeks, not over 2 years. The unusual finding in this study was the significantly lower incidence of total tumors and longer survival in female mice as compared to the controls.

Lindenschmidt, et al. (1986) also found increased incidence of hepatocellular adenomas in a 10 month study using C3H mice. Doses were 0.5% or 0.05% of the diet. The incidence was only significant at the low dose when compared to the lab chow controls but was significant at both levels when compared to the controls on a BHT-free lab diet.

In mechanistic studies, Powell, et al. (1986) found dose-related hepatocellular damage in Wistar rats given 250, 500, 1000 or 1250 ppm BHT (lower doses for up to 28 days, the higher doses given for 4 doses). Kitchen and Brown (1987) found hepatic DNA damage in Sprague-Dawley rats given 700 mg/kg for 2 doses. And, paradoxically, Williams, et al. (1991) found that BHT significantly reduced both benign and malignant liver tumors in AAF initiated F344 rats...but BHT increased the incidence of bladder tumor formation in the F344 rat.

Other tumors:

In addition to the liver tumor findings, lung tumors have been found as a promotional effect in urethan-induced Strain A mice (Witschi, 1984, noted below) and in BALB/c mice with or without the addition of diethylnitrosamine (Clapp, 1973b as mentioned above). Bladder tumors were found to be promoted as noted in the Williams data and by Imaida (1983, found in F344 rats). However, Imaida also found that BHT inhibited the extent of gamma-glutamyl transpeptidase (an enzyme associated with hepatopromoters) induction. Increased DNA synthesis of bladder epithelium was found in male F344 rats by Shibata, et al. (1989). Colon tumors were noted in 1,2-dimethyl-hydrazine (DMH) treated BALB\c mice, but not in those treated with N-nitroso-N-methylurea (MNU).

Witschi, using A/J mice (1984) given BHT at 0.75% of diet before exposing the mice to urethan inhibited lung tumor formation. However, given after exposure to urethan, BHT had a promoting effect on lung tumor development. Dose and duration were factors in the promoting activity of BHT. Two weeks on BHT after urethan dosing provided a significant effect, whereas one week did not. Dose-related increases in effect were seen when BHT was increased from .1% to .75% of the diet.This author notes that "given alone, BHT, has never been found to produce an increased incidence or multiplicity of lung tumors in A/J mice". He speculates that the promoting

effect is due to the ability of BHT to induce cell hyperplasia. In conclusion, he notes that BHT has "...been found to have desirable (protection against chemical carcinogenesis) or undesirable (enhancement or promotion of tumor formation or frank carcinogenesis) properties in a variety of biological systems. If we are concerned about their safety as food additives, then we clearly face a next to impossible choice, simply because we do not have any clues at all whether or not in man, and under present conditions of exposure, the desirable or the undesirable properties of BHT...are more likely to prevail".

Negative bioassays are reported by:

NTP (1979), using F344 rats and B6C3F1 mice. However, lung tumors (adenomas) were increased in female mice but lacked a dose related trend. As a result of this finding and based on positive data by other researchers, authors suggested the compound be considered for retest by the NCI Chemical Selection Working Group.

Deichmann, et al.(1955) found no increase in tumor formation in Wistar rats fed BHT at up to 1% in lard for up to 2 years, nor in mongrel dogs fed up to >24,000 mg/kg for 260 days.

Hirose, et al. (1981) failed to find significantly increased tumor incidence in Wistar rats fed up to 1% BHT for up to 104 weeks.

Shirai, et al., (1982) also failed to find significant increases of tumors in BHT treated B6C3F1 mice fed up to 5000 ppm for up to 96 weeks.

Inhibition:

In addition to the Witschi and Imaida data on inhibition mentioned above, other authors found BHT to have inhibitory effects on tumor development as follows: Tatsuta, et al. (1983) described a protective effect of BHT against gastric cancer initiated by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in Wistar rats. Cohen and coworkers (1986) using outbred female Sprague-Dawley rats, found that long-term exposure to BHT in the diet prior to and following administration of the carcinogen DMBA [57-97-6] resulted in a dose-related inhibition of mammary tumorigenesis and adrenal nodulogenesis.

Mutagenicity data on BHT provides conflicting results. Positive and negative assays have been reported on dominant lethal tests in mice and rats and in Chinese hamster ovary cell studies for chromosomal abnormalities. Ames testing has shown negative results. Positive studies have been reviewed for forward mutation in mouse lymphoma cells; sperm abnormalities in mice and examination of human lymphocyte excision repair synthesis.

As noted above, IARC lists BHT as having limited evidence of carcinogenicity in animals. Their evaluation was based on the findings of Clapp et.al. (1974 & 1978), NTP/NCI (1979), Shirai et.al. (1982), Hirose et.al. (1981), Olsen et.al. (1983 & 1986) and Deichmann et.al. (1955). The additional findings of Inai, et al. (1988) and Lindenschmidt, et al. (1986) lend weight to their conclusion. Although the data do not provide a clear picture of the carcinogenicity of BHT, the hepatocarcinogenic findings cannot be dismissed. Findings of hepatocarcinogenicity have shown positive results in both the sensitive B6C3F1 mouse and in the non-predisposed Wistar rat. The screening level for BHT will be based on the hepatocarcinogenicity of BHT using the work of Inai, et al. (1988) as the primary study due to its recent date, earlier tumor development and adequate duration.

Screening level development:

Male mice with hepatocellular adenomas were the only group significantly increased over controls. Average final body weight of male mice was approximately 32 gm. The doses listed in the Inai study for male mice were 0, 1.64 g/kg body weight/day and 3.48 g/kg b.w./day. These doses are adjusted into study average doses to account for the 12 week observation period following the 104 week dosing as follows:

1.64 g/kg/d = 1640 mg/kg/d * (104/120 weeks) = 1421 mg/kg/d
3.48 g/kg/d = 3480 mg/kg/d * (104/120 weeks) = 3016 mg/kg/d

Incidence of hepatocellular adenomas in male mice surviving to at least week 62 (time of first liver tumor death) were: Controls = 6/32; Low dose = 16/42; High dose = 25/47.

Global 82 output on the above data included:

95% Upper Confidence limit on a risk level of 10^{-6} = $1.516213 * 10^{-6}$
M.L.E dose = $5.4274143509 * 10^{-3}$
Chi-square goodness of fit statistic = $6.696722 * 10^{-3}$ is acceptable

$$q_1^{*animal} = \frac{1.516213 * 10^{-6}}{5.4274143509 * 10^{-3}} = 2.7936 * 10^{-4}$$

$$q_1^{*human} = 2.7936 * 10^{-4} * \sqrt[3]{\frac{70\ kg}{0.032\ kg}} = 3.6264 * 10^{-3}$$

$$q_1^{*}\left(\frac{ug}{m^3}\right)^{-1} = 3.6264 * 10^{-3}\ \left(\frac{mg}{kg}\right)^{-1} * \frac{1mg}{1000ug} * \frac{20\ m^3}{70\ kg} = 1.0361 * 10^{-6}\ \left(\frac{ug}{m^3}\right)^{-1}$$

$$IRSL = \frac{1 * 10^{-6}}{1.0361 * 10^{-6}} = 9.6516 * 10^{-1} = 1 \frac{ug}{m^3} \text{ based on annual averaging}$$

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MLH:ma