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Decrease of Butylated Hydroxyanisole Added in the Diet for a Carcinogenicity Test in Rats and Mice

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Reevaluation of the toxicity and carcinogenicity of food additives is an international pressing needs. When these long term tests are carried out in experimental animals, the test substance is generally added in the diet or the drinking water. In the case of the test using rats or mice, pellet type diet containing a definite concentration of the test substance is often used for its convenience. The outline of the manufacturing process of the pellet type diet is as follows; a definite amount of the test substance is added in the basal powdered diet and mixed well. The mixture is treated by a pelleting machine with steam blowing, and then wet pellets are dried under the hot ventilation. Decrease of the concentration of the test substance is then considered while the manufacturing process of the diet, and there is a fear that the test substance decomposes or some unknown substances are formed.

Prior to a carcinogenicity test of butylated hydroxyanisole (BHA), an antioxidant, we determined BHA in the pellets in which the substance had been added at definite concentrations because of the above aspects, and studied the cause of the decrease of BHA in the diet.

MATERIALS AND METHODS

Reagent and diet. BHA, 3(2)-tert-butyl-4-hydroxyanisole, n-hexane and acetone are reagent grade. The pellet type solid diet added 0, 0.02, 0.1, 0.2, 0.5, 1, and 2% of BHA in the basal diet (CRF-1, Charles River) for rats and mice were prepared by Charles River Japan Inc., Kanagawa.

Heating of BHA. An n-hexane solution containing 50mg of BHA, which corresponds to 5g of the 1% BHA containing diet, was poured into an 85mm-diameter glass dish. The dish was stood at room temperature for the evaporation of the solvent, and was put for a definite time in an electric drying oven maintained at 80°C. BHA remained in the dish was dissolved in n-hexane and the UV absorption was determined at 289nm by a Shimadzu

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UV-200 double beam spectrophotometer.

Sublimation test of BHA. An n-hexane solution containing 50mg of BHA was poured into the sublimation bottle shown in Figure 1. After the solvent was evaporated, the bottle was kept in a water bath maintained at 80°C for 2h, and the bottle and the cold finger were rinsed with n-hexane. n-Hexane in the trap was directly used for the determination of BHA.

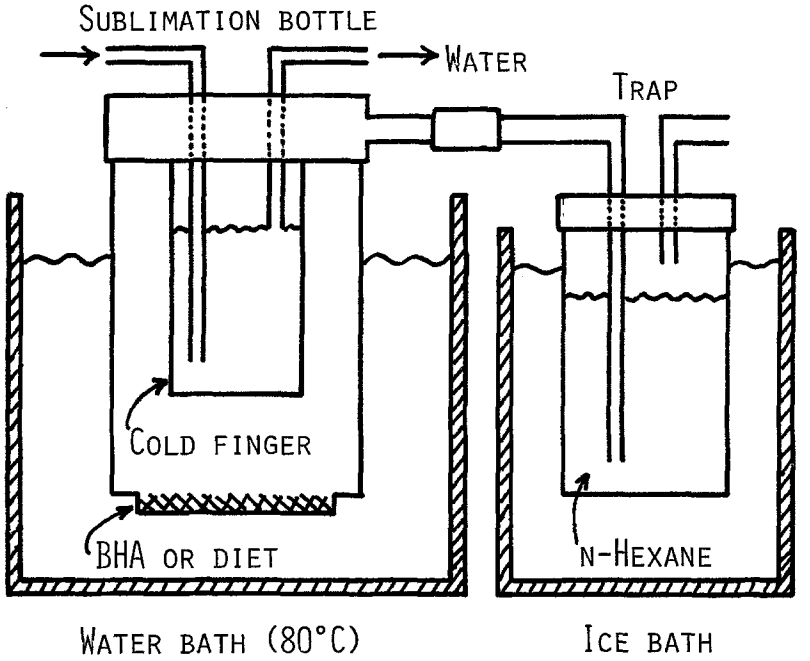

Figure 1. Equipment for sublimation test.

Heating of BHA mixed with the diet. The mixture of 50mg of BHA and 5g of the basal diet which had been previously powdered was spread in the dish and put in the oven (80°C) for a definite time. After heating, BHA was extracted with n-hexane and determined by gas chromatography using a flame ionization detector (PHARMACEUTICAL SOCIETY OF JAPAN 1980). A 1.5m x 3mm i.d. glass column was packed with 5% OV-17 on 60-80 mesh Gas Chrom Q. The instrument was programed as follows: column, 170°C; injection port and detector, 200°C; carrier gas, nitrogen 60mL/min.

Sublimation test of BHA mixed with the diet. The mixture of 50mg of BHA and 5g of the powdered basal diet was put in the sublimation bottle and heated at 80°C for 2h. After heating, BHA in the bottle including the diet and that on the cold finger was extracted with n-hexane, and determined by gas chromatography as described above.

n-Hexane in the trap was directly used for gas chromatography.

Determination of BHA in the diet prepared at a factory. Several pieces of the pellet were powdered by a mixer and weighed the powder. BHA was extracted with n-hexane and determined by gas chromatography as described above.

RESULTS AND DISCUSSION

Contents of BHA in the pellet in which BHA has been added at definite concentrations and which has been heated at 80°C for 2h in the factory are shown in Table 1. Most diets contained less than a half of the indications.

TABLE 1. Determination of BHA added in the pellet type diet for a carcinogenicity test

Indication of BHA content(%)	Repeat of diet manufacture				
	1	2	3	4	5
	(Percent for the indication)				
0.02	15.5	35.0	32.6	39.9	31.4
0.2	35.9	36.0	29.7	30.0	21.7
0.5	56.6	38.4	49.7	42.6	52.8
1	60.0	57.7	48.8	54.6	
2	64.3	39.3	55.4	52.1	56.2

Although BHA is known as one of sublimable substances, there are possibility of the oxidation and decomposition it self or the reaction with the diet components during the heating process because of its reactivity (ROSENWALD and CHENICEK 1951, ISHITANI et al. 1976).

When BHA alone was heated at 80°C, decrease of BHA was very rapid and only 14.9±4.6% of BHA remained at 2h, and 70.3±2.3% remained when BHA was heated with the diet. Half lives of BHA in these conditions were 1.0h for BHA alone and 3.4h for the mixture of BHA and the diet, respectively (Fig. 2). These results showed that BHA in the pellet had decreased while the manufacturing process.

When BHA alone was heated in the sublimation bottle, 55.6±3.2% was remained in the bottle and 41.9±4.1% of BHA was sublimated and recovered from the cold finger, and 89.8±1.6% of BHA remained in the bottle and 6.1±1.1% was recovered from the cold finger when BHA was mixed with the diet (Table 2). No UV absorption and no peaks on the gas chromatogram were observed in n-hexane in the trap.

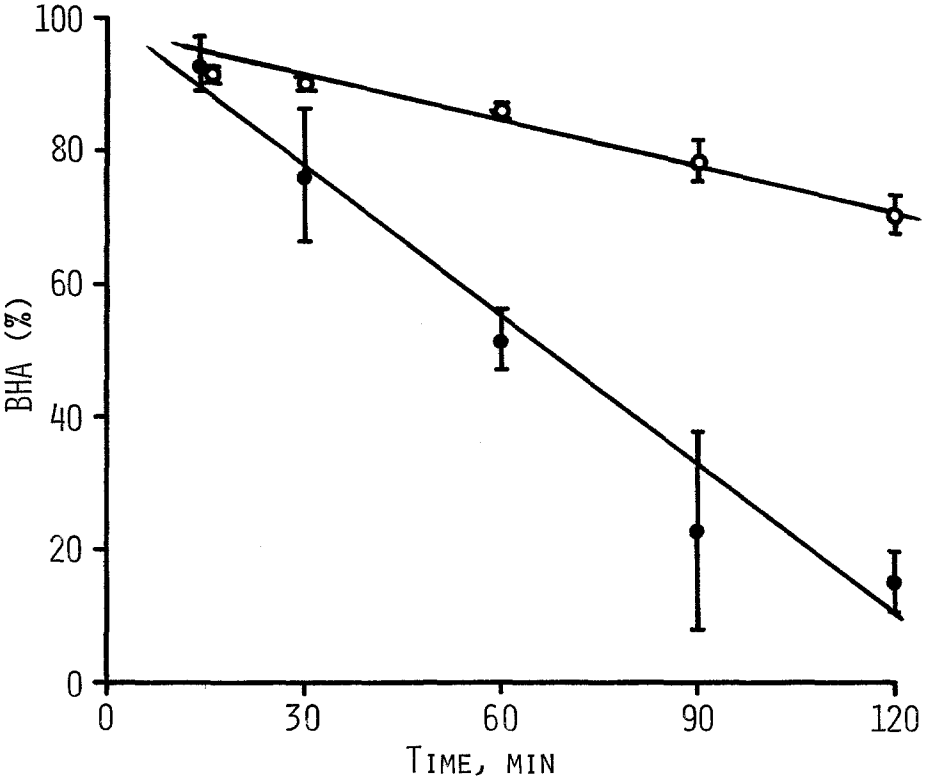

Figure 2. Decrease of BHA by heating at 80°C, mean ± S. D. (n=3).

TABLE 2. Sublimation tests of BHA alone and mixed with diet

	BHA alone (%)	Mixed with diet (%)
Bottle	55.6 ± 3.2	89.8 ± 1.6
Cold finger	41.9 ± 4.1	6.1 ± 1.1
Trap	N.D.	N.D.
Total	97.5 ± 1.5	96.0 ± 0.6

mean ± S.D.

The dish and the sublimation bottle, which had been rinsed with n-hexane, was rinsed with a small portion of acetone. But the acetone solution gave no UV absorption or no peak on gas chromatogram. Through the experiments, the n-hexane extracts showed no change of the UV spectra and no peaks except the peak of BHA on gas chromatograms (Fig. 3a and b).

In the present experiments, ventilation was not

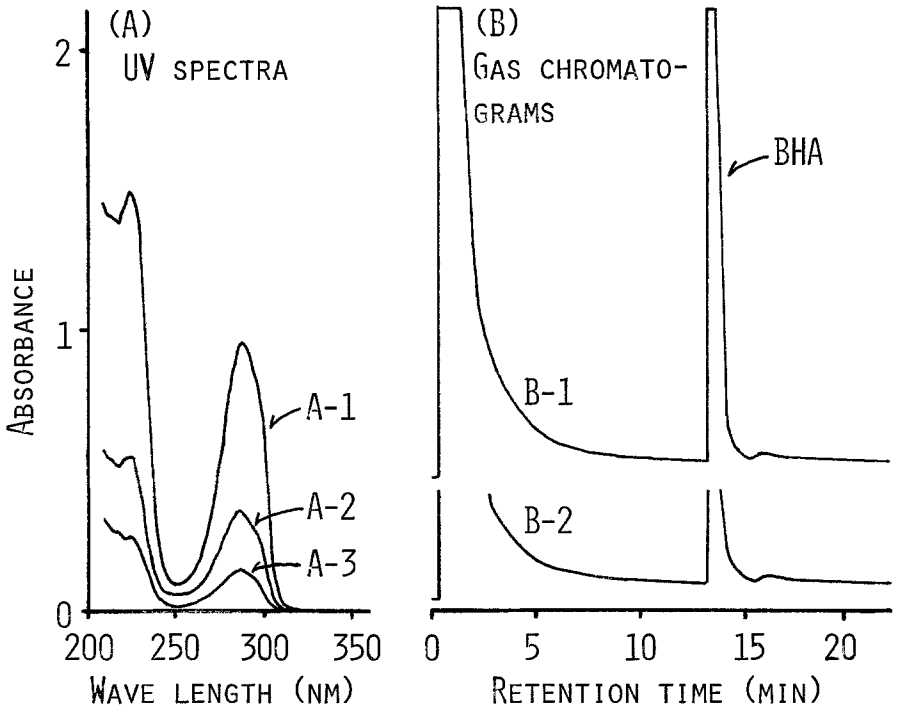

Figure 3a and b. UV spectra (a) and gas chromatograms (b) of BHA heated at 80°C.
A-1 and B-2, 0h; A-2, 1h; A-3 and B-1, 2h.
Column temperature on gas chromatography was from 100 to 190°C (program rate, 5°C/min).

carried out, then, higher decrease ratio is presumed by hot ventilation such as manufacturing process. From these results, it is considered that the main cause of the decrese of BHA in the pellet type diet was sublimation while the heating process including the steaming.

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