Bull. Environ. Contam. Toxicol. (1984) 33:533–537

Environmental Contamination and Toxicology

Detection of 4-Hydroxy-BHT Residues in Laboratory Animals as an Indicator of Exposure to Butylated Hydroxytoluene (BHT)

Kenji Yamamoto,[1] Kazuo Tajima,[1] and Tamio Mizutani[2]

[1]School of Pharmacy, Hokuriku University, Kanazawa 920-11, Japan, and
[2]Faculty of Living Science, Kyoto Prefectural University, Kyoto 606, Japan

Butylated hydroxytoluene (BHT) is a phenolic antioxidant used widely in processed foods and petroleum products. BHT tends to accumulate in animal tissues rich in lipids during the daily intake (Daniel and Gage 1965; Gilbert and Golberg 1965). Its accumulation in human adipose tissues was found by colorimetry (Collings and Sharratt 1970) and by gas chromatography with an electron-capture detector (GC-ECD) after being derivatized (Mizutani and Ohe 1976).

In a previous study (Mizutani et al. 1983) dealing with the metabolism of BHT in mice, we analyzed 2,6-di-tert-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone (4-hydroxy-BHT), a metabolite of BHT, at nanogram levels by GC-ECD. A peak having the same retention time as 4-hydroxy-BHT appeared often on gas chromatograms of the liver and lung extracts from untreated mice. This peak was identified as 4-hydroxy-BHT by thin-layer chromatography, gas chromatography, and gas chromatography-mass spectrometry (GC-MS) with selected ion monitoring. This paper deals with the identification and determination of 4-hydroxy-BHT residues in the tissues of untreated laboratory animals.

MATERIALS AND METHODS

4-Hydroxy-BHT was synthesized by the described method (Kharasch and Joshi 1957).

Male ddY mice and male Wistar rats (Sankyo Labo Service Co., Tokyo, Japan), 4 weeks of age, were used. Mice were housed in plastic cages on a wood chip bedding (White Flake, Charles River Japan, Inc., Kanagawa, Japan), and rats in stainless steel wire cages. The animals received ad libitum a standard laboratory diet (Funabashi F-2, Funabashi Farms, Chiba, Japan) and water for the periods scheduled as shown in Table 1.

The livers and lungs were excised and homogenized in 1.15% KCl (3 ml/g of tissue), and the homogenate was extracted with benzene (5 ml/g of tissue). The extract was evaporated to dryness and chromatographed on a silica gel plate (Silica gel HF 254, E. Merck A.G., Darmstadt, Germany) with a solvent mixture of methanol and benzene (2 : 98). The area corresponding to 4-hydroxy-BHT was scrapped off and extracted with benzene. The extract was subjected to GC-ECD and GC-MS with selected ion monitoring. For quantitative analysis, the extracts from the liver and lung homogenates were subjected to GC-ECD without purification by thin-layer chromatography.

GC-ECD was performed on a Hitachi 164 gas chromatograph fitted with a 2 m X 3 mm i.d. glass column packed with Chromosorb W containing 2% OV-1. 4-Hydroxy-BHT was analyzed at 130°C using nitrogen (60 ml/min) as carrier gas. GC-MS was performed on a JEOL JMS-D 100 GC-MS spectrometer equipped with a JEOL MS-PD-01 multiple ion detector. The ionizing energy was 22 eV. The selected ion monitor was focused on the ions m/z 165, 180, and 193 for 4-hydroxy-BHT.

RESULTS AND DISCUSSION

Figure 1 (A) shows the typical gas chromatogram of the liver extract from untreated mice. The component present in the chromatogram was identified as 4-hydroxy-BHT by gas chromatographic and thin-layer chromatographic comparison with the authentic sample.

4-Hydroxy-BHT levels in the liver and lung extracts were too low to be identified from its mass spectral fragmentation pattern obtained by GC-MS. Therefore, selected ion monitoring was developed to analyze 4-hydroxy-BHT. Figure 1 (B) shows the selected ion monitoring trace of the mouse liver extract. The component in the chromatogram was confirmed to be 4-hydroxy-BHT by comparison of its GC retention times and responses on the ions m/z 165, 180, and 193 with those of the authentic sample.

ECD exhibited a high sensitivity toward 4-hydroxy-BHT possibly because of its conjugated structure. It has been reported that quinones, 1,2-diketones, and pyruvate esters are determined by GC-ECD at nanogram levels (Gudzinowicz 1967). The response for 4-hydroxy-BHT was linearly related to amount in the range of 0.01 - 0.1 ng. The recovery of 4-hydroxy-BHT added to the mouse liver homogenate was 87 - 92%. Table 1 shows the

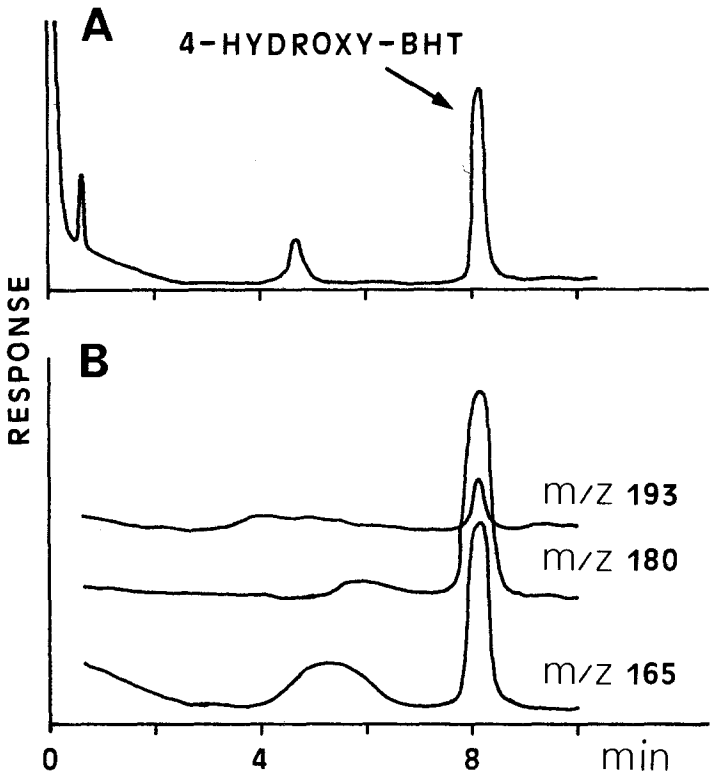

Figure 1. GC-ECD chromatogram (A) and selected ion monitoring trace (B) of the liver extract from untreated mice on an OV-1 column.

concentrations of 4-hydroxy-BHT in the livers and lungs of untreated mice and rats at various times after feeding a standard diet. These data were uncorrected with the recovery of 4-hydroxy-BHT.

It has been shown that BHT is metabolized to 4-hydroxy-BHT via 2,6-di-tert-butyl-4-hydroperoxy-4-methyl-2,5-cyclohexadienone in vitro (Shaw and Chen 1972) and in vivo (Yamamoto et al. 1979). Therefore, it seems likely that 4-hydroxy-BHT found in laboratory animal tissues is formed from BHT by metabolic reactions and subsequently accumulated. BHT is used as an antioxidant in many types of foods and feeds such as fats, oils, milk products, and fish products. It is also used as a stabilizer in rubber products, plastics, industrial oils, and synthetic fibers. Its use as an additive in feeds and probable broad distribution in environments leave no doubt that laboratory animals are more or less

Table 1. 4-Hydroxy-BHT concentrations in mice and rats at various times after feeding a standard diet, mean ± SE of four determinations, ng/g of wet tissue

Animal	Feeding period (days)	Liver	Lung
Mice			
	7	8.0 ± 0.5	8.6 ± 0.4
	42	8.6 ± 0.2	11.0 ± 0.4
	77	8.7 ± 0.2	12.3 ± 0.5
Rats			
	7	11.2 ± 2.1	14.2 ± 3.0
	28	15.2 ± 0.6	19.8 ± 1.3

contaminated with BHT. Moreover, the administration of olive oil used as a vehicle to mice increased 4-hydroxy-BHT concentrations in the liver and lung tissues as compared with untreated mice (unpublished data).

BHT has been shown to increase microsomal enzyme activities in mice and rats (Gilbert and Golberg 1965). Therefore, BHT contamination in diets and vehicles may influence the metabolism and toxicity of drugs in laboratory animals.

BHT in foods is usually determined by gas chromatography with a flame ionization detector at microgram levels. The sensitivity of this method is too low to determine BHT in animal materials. Although the metabolic rate of BHT to 4-hydroxy-BHT in vivo after administration of BHT in low levels has not been studied, the analysis of 4-hydroxy-BHT by GC-ECD provides a sensitive indicator of animal exposure to BHT.

REFERENCES

Collings AJ, Sharratt M (1970) The BHT content of human adipose tissue. Food Cosmet Toxicol 8:409-412

Daniel JW, Gage JC (1965) The absorption and excretion of butylated hydroxytoluene (BHT) in the rat. Food Cosmet Toxicol 3:405-415

Gilbert D, Golberg L (1965) Liver response tests. III. Liver enlargement and stimulation of microsomal

processing enzyme activity. Food Cosmet Toxicol 3: 417-432

Gudzinowicz BJ (1967) Gas chromatographic analysis of drugs and pesticides. M. Dekker, New York, pp. 512-537

Kharasch MS, Joshi BS (1957) Reactions of hindered phenols. II . Base-catalyzed oxidations of hindered phenols. J Org Chem 22:1439-1443

Mizutani T, Ohe T (1976) Hygienic chemical studies on 3,5-di-tert-butyl-4-hydroxytoluene (BHT). II . Determination of 3,5-di-tert-butyl-4-hydroxytoluene (BHT) in human adipose tissue. Eisei Kagaku 22:265-270

Mizutani T, Yamamoto K, Tajima K (1983) Isotope effects on the metabolism and pulmonary toxicity of butylated hydroxytoluene in mice by deuteration of the 4-methyl group. Toxicol Appl Pharmacol 69:283-290

Shaw Y-S, Chen C (1972) Ring hydroxylation of di-t-butylhydroxytoluene by rat liver microsomal preparations. Biochem J 128:1285-1291

Yamamoto K, Tajima K, Mizutani T (1979) Identification of new metabolites of butylated hydroxytoluene (BHT) in rats. J Pharm Dyn 2:164-168

Received January 30, 1984; Accepted March 7, 1984