Confirming Low Levels of Hexabromobiphenyl by Gas-Liquid Chromatography of Photolysis Products

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INTRODUCTION

fireMaster ®BP-6 (BP-6) (Michigan Chemical Co.), a commercially produced hexabromobiphenyl (HBB), has been used extensively to retard the flammability of thermoplastics, e.g., business machine and electrical housings. BP-6 is a mixture of the following brominated biphenyls: tetra-, 2%; penta-, 10%; hexa-, 63%; hepta-, 14%; and other bromobiphenyls, 11% (KERST, 1974). Studies using ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy have identified the principal component as 2,2',4,4',5,5'-HBB (ANDERSSON et al., 1974; SUNDSTROM and HUTZINGER, 1976). Identification and quantitation of residues resulting from contamination with BP-6 generally have been based upon comparison of the gas-liquid chromatographic (GLC) peak due to the HBB homolog of the residue with that of a BP-6 reference. ERNEY (1975) reported a photolysis method for confirming HBB residue identity, demonstrating applicability with extracts of Swiss cheese fortified with 0.33 ppm BP-6 on a fat basis. An ultraviolet (UV) viewing box for thin layer chromatographic plates was used to photolyze the sample solutions. The cheese extract in petroleum ether solution was passed through a micro-Florisil column with 15 ml 6% ethyl ether-petroleum ether eluant. Gas-liquid chromatograms of the BP-6 solutions showed that for levels of HBB residues quantitated at about 0.1 ppm or less (fat basis) BP-6, improved sample cleanup would be needed to use photolysis for confirmation. It was found (FARRELL, 1976) that BP-6 can be quantitatively eluted from a Florisil column (OFFICIAL METHODS OF ANALYSIS, 1975; PESTICIDE ANALYTICAL MANUAL, 1973) with 150 ml petroleum ether. This investigation was directed toward the development of a method for confirming the identity of HBB residues quantitated at about 0.1 ppm BP-6 or less in food commodities. Important procedural requirements were the attainment of adequate cleanup of sample extracts and GLC resolution so that BP-6 and its initial photoproducts could be detected by the electron capture (EC) detector without interfering peaks. We also wished to elucidate the polybrominated biphenyl photochemistry involved in the confirmatory technique.

EXPERIMENTAL

Reagents and Apparatus

(a) Florisil.-PR grade, 60-100 mesh (Floridin Co., Berkeley Springs, WV 25411). If received in plastic containers or paperboard boxes, transfer Florisil to glass containers with glass-stoppered or foil-covered lids and store in dark. Heat at 130°C for $\geq$5 hr but preferably overnight before use. Store at 130°C in foil-covered bottles.

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(b) Solvents.-Hexane (UV cut-off, <200 nm) and petroleum ether (UV cut-off, 218 nm) suitable for use in pesticide residue analysis, distilled-in-glass (Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442), or equivalent.

(c) Photoreactor.-Rayonet (Southern New England Ultraviolet Co., Middletown, CT 06457) Model RPR-100. The reactor was equipped with Rayonet Merry-Go-Round Model MGR-100 which operated at 5 rpm; 3 ml solutions were photolyzed at ambient temperature (25°C) in 1 cm diameter cylindrical Rayonet Model RQV-7 quartz cells. Four Rayonet Model RPR-2537Å lamps set at 90° angles to each other were used in the reactor. To obtain the initial photoproducts of BP-6, the 4 lamps were partially covered with black tape with 1-2 mm of each lamp exposed. The part of each lamp exposed was that in closest proximity to the photolyzed solution. The light intensity incident on a 3 ml solution in a cell was calculated (HATCHARD and PARKER, 1956) to be 2×10^{14} quanta/sec.

(d) Gas chromatograph.-Searle-Analytic (Des Plaines, IL 60018) Model 5360 with 6' x 4 mm id glass column packed with 1% OV-101 on 80-100 mesh Chromosorb W(HP). Operating conditions: column flow, 60 ml N_2/min; column, 202°C; detector, 212°C; injector, 225°C; detector, pin-cup design EC with titanium ^3H foil; dc detector voltage adjusted to cause one-half scale deflection for the HBB homolog peak with 1.4 ng BP-6 injected when full-scale deflection was 1×10^{-9} amp. For greater sensitivity the detector voltage was adjusted to cause one-half scale deflection for the HBB peak with 0.36 ng BP-6 injected when full-scale deflection was 1×10^{-9} amp.

(e) Spectrophotometer.-Cary (Varian Analytical Instrument Division, Palo Alto, CA 94303) Model 14 equipped with 1 cm path length quartz cells. Operating conditions: 5Å/sec; hexane in reference cell.

(f) Mass spectrometer.-Finnigan (Sunnyvale, CA 94086) Model 3300F, coupled to Finnigan 9500 gas chromatograph via glass jet separator. GLC operating conditions: 6' x 2 mm id glass column containing 3% OV-101 on 80-100 mesh Supelcoport; column flow, 26 ml He/min; injector, 270°C; column, temperature programed between 230° and 270°C at 4°C/min. Mass spectrometer operating conditions: electron energy, 70 ev; emission current, 500 μa; multiplier voltage, 1.8 kv.

Method

The sample solution to be photolyzed must not contain traces of any solvent which can render the solution opaque, e.g., benzene, or solvent which can undergo photoreaction other than hydrogen donation (halogenated solvents can possibly donate halogens). Gradual formation of initial photoproducts with concomitant

diminution of HBB may be observed by GLC analysis of BP-6 solutions photolyzed for various increments of time. Shielding of high intensity photolysis lamps by physical barriers, such as black adhesive tape, may be necessary to control the photolysis rate.

Extract and clean up sample containing suspected HBB residue according to OFFICIAL METHODS OF ANALYSIS (1975) or PESTICIDE ANALYTICAL MANUAL (1973) methods, substituting 150 ml petroleum ether as the only eluant for Florisil column chromatography. For previously cleaned up extracts eluted with 200 ml 6% ethyl ether-petroleum ether, re-chromatograph extract through additional Florisil column with 150 ml petroleum ether eluant. Concentrate or dilute petroleum ether eluate to suitable volume for GLC analysis. Obtain gas chromatograms of unphotolyzed sample and BP-6 petroleum ether reference solution of similar concentration. Use 3 ml or other suitable aliquots of these solutions for subsequent photolysis. Photolyze BP-6 reference solution to determine appropriate lamp exposure and duration of photolysis required to obtain adequate GLC detection of initial photoproducts of BP-6. Use quartz cells and 2537Å or other short wavelength ($\leq$3000Å) lamps for photolysis. Obtain gas chromatogram of photolyzed reference solution showing initial photoproducts and diminished HBB peak. Photolyze same volume of sample solution for the same time as in photolysis of reference and compare gas chromatogram of sample's initial photoproducts with that of reference. Confirm HBB residue by demonstrating same GLC retention times of initial photoproducts and diminution of HBB peak in chromatograms of both reference and sample.

RESULTS AND DISCUSSION

The initial investigation of identity confirmatory techniques for HBB residues included attempts to chlorinate BP-6 with antimony pentachloride and to hydrolyze BP-6 with potassium hydroxide. The experimental conditions were identical to those reported by ARMOUR (1973) for perchlorinating polychlorinated biphenyls (PCBs) and by YOUNG and BURKE (1972) for potassium hydroxide hydrolysis. GLC of BP-6 after attempted chlorination and after potassium hydroxide treatment showed erratic degradation of HBB. These limited experiments indicated that these techniques are not applicable to forming a characteristic derivative for confirming the identity of HBB residues. UV photolysis was then tried as an identity confirmation method.

Photolysis of BP-6 Reference Solution

BP-6 exhibits a UV absorption maximum in hexane at 230 nm (absorptivity, 5.2×10^{-2} ml $\mu g^{-1} cm^{-1}$). BP-6 solutions were photolyzed at 2537Å (absorptivity, 1.3×10^{-2} ml $\mu g^{-1} cm^{-1}$ in hexane). BP-6 hexane and petroleum ether solutions in Pyrex vessels were stable when exposed to normal laboratory fluorescent lighting for several weeks. Identical results were obtained when petroleum

ether or hexane solutions of BP-6 were photolyzed. Figure 1 shows the gas chromatograms of a 97 ng BP-6/ml petroleum ether solution, with no sample extract present, before and after 10 and 48 min photolysis. Peak 1 was determined by GLC-mass spectrometric (MS) analysis to be due to the HBB homolog. Peaks 2 and 3, with retentions of 0.41 and 0.45, respectively, relative to the HBB homolog (RR_{HBB}), are the dominant increasing peaks during the initial photolysis period. Peaks 2 and 3 were determined by GLC-MS analysis to be due to pentabromobiphenyls. After 48 min photolysis, the peaks at RR_{HBB} of 0.41 and 0.45 and the HBB peak were essentially eliminated. Two small peaks with RR_{HBB} values of 1.6 and 2.6 and peak heights of 0.03 and 0.12, respectively, relative to the HBB homolog before photolysis, were present in the gas chromatograms of unphotolyzed BP-6 solutions. These peaks have long retention times. Therefore, the chromatograms in Fig. 1 and the subsequent Fig. 2 do not include the 2 late-eluting peaks; these peaks were essentially eliminated by photolysis.

Photolysis of BP-6 in Cleaned Up Sample Extracts

For this study food samples were extracted and cleaned up according to the methods of the PESTICIDE ANALYTICAL MANUAL (1973) except that 150 ml petroleum ether was used as the only eluant for the Florisil column chromatographic cleanup of extracts. Cleaned up extracts representing 3 g butter (fat basis), 3 g Graddost cheese (fat basis), and 15 g eggs (whole basis) were fortified with 0.05, 0.05, and 0.005 ppm BP-6, respectively, prior to photolysis. Figure 2 shows gas chromatograms of the petroleum ether eluate of the butter extract fortified with BP-6 and the sample blank, each before and after 11 min photolysis. After about 11 min photolysis, Peak 2 (RR_{HBB} of 0.41) was significantly larger than Peak 1 (HBB homolog). With further photolysis, Peaks 1 and 2 were essentially eliminated. Little change in the GLC peaks due to the sample extract was observed during photolysis. The gas chromatograms of the BP-6 fortified and blank extracts of the Graddost cheese and eggs before and after 13 min photolysis showed similar applicability of the method. The chromatograms of photolyzed extracts of cleaned up food samples, fortified with 0.05 and 0.005 ppm BP-6, provide evidence that sample cleanup and GLC resolution are adequate for confirming the identity of HBB quantitated at about 0.1 ppm BP-6 and below. Florisil column chromatography provides adequate sample cleanup for EC-GLC detection of HBB and the principal initial photoproducts of BP-6. When the GLC parameters described above are used, the initial photoproducts (isomers of pentabromobiphenyl) are more easily detected than secondary photoproducts of BP-6. Further debromination will produce brominated biphenyls which have less EC-GLC response and more vapor pressure. With $\leq$0.1 ppm BP-6, the more volatile photoproducts may be difficult to resolve from early-eluting sample co-extractives in GLC analysis. Butter and Mozzarella cheese samples containing 2.8 and 6.4 ppm HBB, as BP-6 (fat basis),

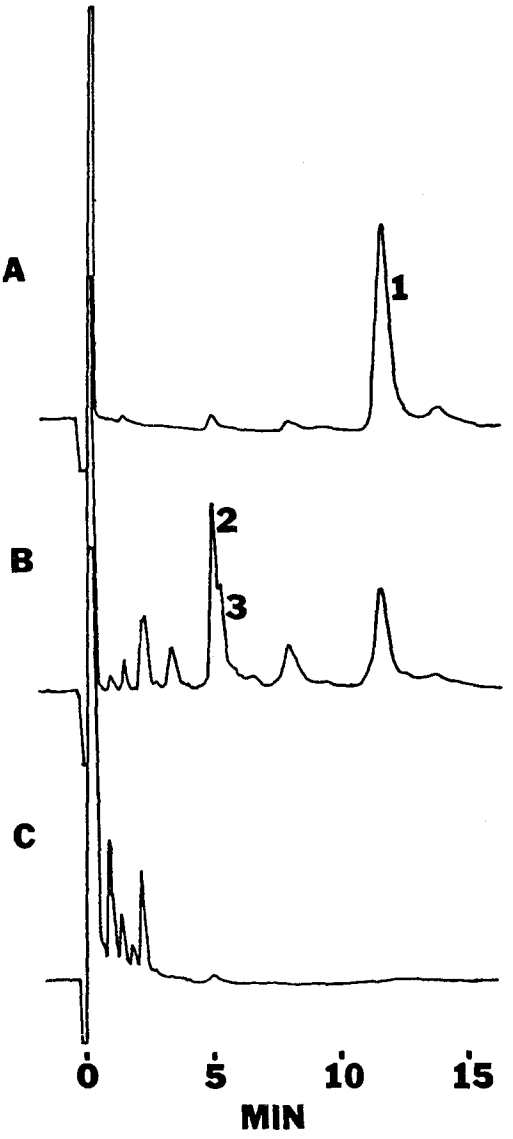

FIG. 1-Electron capture GLC curves of 97 ng/ml BP-6 petroleum ether solution, with no sample extract present, before and after 10 and 48 min photolysis (detector voltage adjusted to cause one-half scale deflection for the HBB peak with 1.4 ng BP-6 injected when full scale deflection is 1 x 10^{-9} amp): A, before photolysis, 1.4 ng BP-6 injected, Peak 1 due to HBB; B, after 10 min photolysis, 2.6 ng equivalent BP-6 injected, Peaks 2 and 3 due to pentabromobiphenyls; C, after 48 min photolysis, 3.0 ng equivalent BP-6 injected.

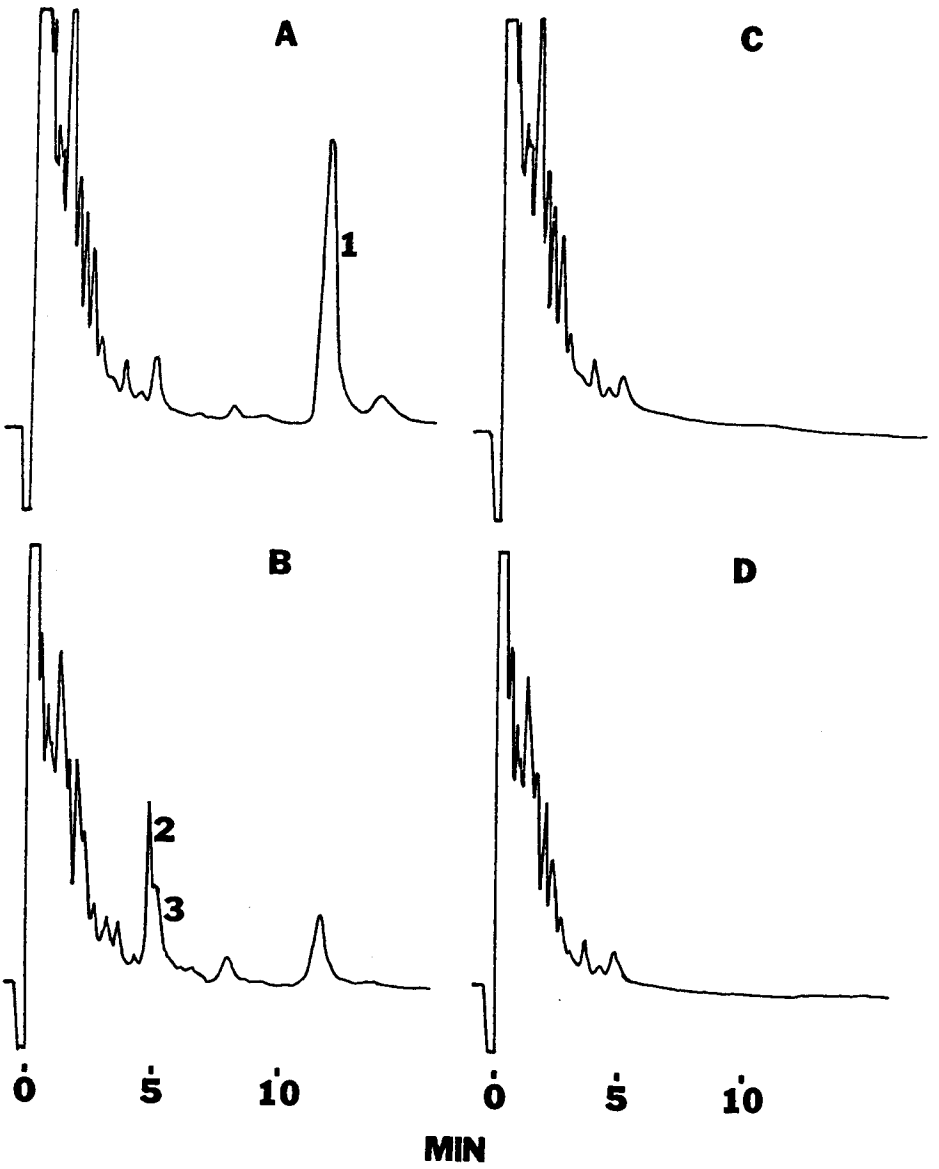

FIG. 2-Electron capture GLC curves of petroleum ether eluate of butter extract, fortified with 0.05 ppm BP-6 (fat basis) and sample blank, before and after 11 min photolysis (detector voltage adjusted to cause one-half scale deflection for the HBB peak with 0.36 ng BP-6 injected when full scale deflection is 1 x 10^{-9} amp): A, fortified eluate before photolysis, 10 mg equivalent butter fat injected, Peak 1 due to HBB; B, fortified eluate after 11 min photolysis, 12 mg equivalent butter fat injected, Peaks 2 and 3 due to pentabromobiphenyls; C, sample blank before photolysis, 11 mg equivalent butter fat injected; D, sample blank after 11 min photolysis, 11 mg equivalent butter injected.

respectively, were photolyzed for 10 and 13 min, respectively. The chromatograms of these samples before and after photolysis showed the applicability of the described method for confirming the identity of actual HBB residues in food. For relatively clean extracts containing HBB residues, the method reported by ERNEY (1975) may be used. Contamination of laboratory glassware and equipment by HBB should be avoided. GLC peaks due to BP-6 and its photoproducts were observed after photolysis in some sample blanks. This was caused by contamination from previous use of the cells for BP-6 photolysis. Cleaning glassware with a saturated solution of potassium dichromate in concentrated sulfuric acid and rinsing with distilled water followed by acetone and petroleum ether eliminated this problem.

The photoreactor with the lamp emission modification was adequate to obtain confirmatory photoproducts of BP-6 in petroleum ether and hexane. The photodisappearance quantum yield (number of molecules reacted per quanta absorbed) of BP-6 in hexane (measured by the decrease in the GLC peak of the HBB homolog) was calculated (HATCHARD and PARKER, 1956) to be 0.26 at 2537Å. The highly efficient photoreactivity of BP-6 may be explained by one of the following: (1) enhanced inter-system crossing to a triplet state due to vibronic coupling with the bromines, (2) steric interference due to the bromines, and (3) the relatively low carbon-bromine bond energy. The geometry of triplet biphenyl has been shown to be planar (WAGNER, 1967). Ortho substituents shift the phosphorescent emission of biphenyl to higher energies (LEWIS and KASHA, 1944) presumably because of steric crowding in the planar excited state. HBB in the excited state could be expected to possess steric interference due to 6 bromines, especially those in the ortho positions. RUZO et al. (1974) have shown that for PCBs upon photoexcitation, ortho-chlorines preferentially cleave. With PCBs containing only meta- and para-chlorines, meta-chlorines are lost preferentially upon irradiation. It is suggested that 2,2',4,4',5,5'-HBB, the major component of BP-6, preferentially loses an ortho-bromine upon irradiation to yield 2,3',4,4',5-pentabromobiphenyl and to a lesser degree loses a meta-bromine to yield 2,2',4,4',5-pentabromobiphenyl. It is further suggested that Peaks 2 and 3 (Figs. 1 and 2) are due to the latter 2 compounds, respectively. RUZO and ZABIK (1975) have found that BP-6 photoreacts 7 times as fast as its chlorinated counterpart, 2,2',4,4',5,5'-hexachlorobiphenyl. They attribute the increase in reactivity to the lower carbon-bromine bond energy [aromatic C-Br bond, 71 kcal/mole vs. aromatic C-Cl bond energy, 86 kcal/mole (KERST, 1974)].

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