Synthesis and Identification of Highly Toxic Polybrominated Biphenyls in the Fire Retardant FireMaster BP-6

Larry W. Robertson, Stephen H. Safe,* Andrew Parkinson, Edo Pellizzari, Carol Pochini, and Michael D. Mullin

The identity of over 91% of the polybrominated biphenyl (PBB) components present in fireMaster BP-6 was determined by capillary gas chromatography (GC) and GC–mass spectrometry (MS) with 22 individual PBB congeners as reference standards. There was an excellent correlation between the observed retention times of the individual PBBs (and of the corresponding GC peaks in fireMaster) and the expected retention times calculated from the degree of bromination and substituent orientation for the PBB standards. Previous studies indicate that the more toxic PBBs are also inducers of rat hepatic microsomal aryl hydrocarbon hydroxylase (AHH). This paper reports the GC and GC–MS identification of seven additional PBBs in fireMaster that also induce aryl hydrocarbon hydroxylase. Included in this group of seven potentially toxic PBBs are the coplanar 3,3′,4′,4′-tetra-, 3,3-,4,4-,5-penta-, and 3,3-,4,4-,5,5′-hexabromobiphenyls, which represent the most toxic group of PBB congeners identified in fireMaster.

The accidental substitution of fireMaster, a commercial polybrominated biphenyl (PBB) flame retardant, for nutriMaster, a magnesium oxide cattle feed supplement, resulted in a major pollution disaster that was primarily confined to the State of Michigan (Robertson and Chynoweth, 1975; Kay, 1977). The initial contamination of cattle and related agricultural products (Jackson and Halbert, 1974) ultimately resulted in the widespread contamination of the food chain within the state (DiCarlo et al., 1978). PBB residues have been detected not only in the more heavily exposed farm families and industrial workers but also in the general human population of Michigan (Eyster et al., 1983; Brilliant et al., 1978). In common with other halogenated aromatic contaminants, such as the polychlorinated biphenyls (PCBs), PBBs are stored in adipose tissue and have been identified in human blood, fat, and breast milk (Eyster et al., 1983).

Like other toxic halogenated aromatics, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Poland and Knutson, 1982; Poland and Glover, 1980), commercial PBBs cause weight loss, thymic atrophy, liver damage, endocrine disorders, and various skin lesions in exposed animals (Gupta and Moore, 1979; Gupta et al., 1981, 1983a,b). Commercial PBBs are also potent inducers of a variety of drug-metabolizing enzymes, including several forms of cytochrome P-450 (Dent et al., 1976, 1977, 1978; Parkinson et al., 1983; Dannan et al., 1983). FireMaster can apparently act as both a genotoxic and epigenetic carcinogen. Administration of the commercial PBB to rats causes hepatocellular carcinomas and enhances the carcinogenicity of diethylnitrosamine (Kimbrough et al., 1981; Jensen et al., 1982), whereas topical application of the PBB mixture to hairless mice pretreated with specific carcinogens results in skin tumor promotion (Poland et al., 1982).

The human health impact of PBBs has not been delineated. Workplace exposure to this chemical is suggested as a likely cause of hypothyroidism in the workers surveyed (Bahn et al., 1980). It has also been suggested that neonatal exposure to PBBs may hinder the neurophysiological development of young children, although the significance of this conclusion has been questioned (Seagull, 1983; Schwartz and Rae, 1983). A major problem associated with determining the potential short- and long-term health effects of PBBs is related to the complex composition of the commercial product. Although the structures of some of the major PBB congeners in fireMaster have been delineated (Moore and Aust, 1978; Moore et al., 1980; Hass et al., 1978), there are several unknown minor components that are suspected of playing a major role in the toxicity of the mixture. Like the PCBs (Safe et al., 1982) and related halogenated aromatics, the most toxic PBB isomers and congeners are substituted in both para and one or more meta positions of the biphenyl ring (Robertson et al., 1982) and as such are approximate isostereomers of 2,3,7,8-TCDD. The identification and quantitation of these compounds and other toxic PBBs in fireMaster are a major synthetic and analytical task due to the lack of authentic PBB standards and their synthetic precursors. This paper describes the synthesis of 19 PBBs and the identification by high-resolution capillary GC and GC–MS of several new PBB components in fireMaster BP-6. We provide evidence for the presence of several previously unidentified toxic PBBs in this commercial mixture.

EXPERIMENTAL SECTION

Chemicals and Biochemicals. 4-, 3-, and 2-bromoaniline, 2,4-dibromoaniline, 2,5-dibromoaniline, 2,6-dibromo-4-nitroaniline, 1,2,4,5-tetrabromobenzene, 1,2-dibromobenzene, and 1,4-dibromobenzene were purchased from Aldrich Chemical Co., Milwaukee, WI. Amyl nitrate, 1,2,4-tribromobenzene, and 3,4-dibromoaniline were purchased from Pfaltz and Bauer, Stamford, CT. 2,4,5- and 3,4,5-tribromoaniline, 2.3,4,5-tetrabromoaniline, and 1,2,3-tribromobenzene were prepared as previously described (Robertson et al., 1980, 1981a, 1982). 2,2′,4,4′,5,5′-Hexabromobiphenyl was isolated from fireMaster BP-6 by alumina column chromatography as previously reported (Safe et al., 1978). 3,3′,4,4′,5,5′-Hexabromobiphenyl was prepared from 3,3′,5,5′-tetrabromobenzidine as described (Sundstrom et al., 1976). 2,2′,3,4-,5′,6-Hexa-, 2,2′,3,3′,4,4′,5-hepta, and 2,2′,3,4,4′,5,5′-heptabromobiphenyl samples were provided by Dr. S. D. Aust, Michigan State University, East Lansing, MI. All other PBBs were prepared by the diazo coupling of a brominated aniline with excess of a bromi-

Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843 (L.W.R. and S.H.S.), Department of Biochemistry and Drug Metabolism, Hoffman-La Roche Inc., Nutley, New Jersey 07110 (A.P.), Research Triangle Institute, Research Triangle Park, North Carolina 27709 (E.P.), and U.S. Environmental Protection Agency, Large Lakes Research Station, Grosse Ile, Michigan 48138 (C.P. and M.D.M.).

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Table I. PBB Congeneric Standards: Synthesis and Characterization

structure [molecular ion (M^+)]	presursor(s) or source	^1H NMR data or reference (GC purity)
2,2′,5-tri (388)	2-bromoaniline, 1,4-dibromobenzene	Sundstrom et al. (1976) (99%)
2,3′,5-tri (388)	3-bromoaniline, 1,4-dibromobenzene	Sundstrom et al. (1976) (99%)
2,4′,5-tri (388)	4-bromoaniline, 1,4-dibromobenzene	Sundstrom et al. (1976) (99%)
3,4,4′-tri (388)	4-bromoaniline, 1,2-dibromobenzene	Robertson et al. (1982a) (99%)
2,2′,4,5′-tetra (466)	2,4-dibromoaniline, 1,4-dibromobenzene	Sundstrom et al. (1976) (98%)
2,2′,5,5′-tetra (466)	2,5-dibromoaniline, 1,4-dibromobenzene	Robertson et al. (1983) (99%)
2,3′,4,4′-tetra (466)	2,4-dibromoaniline, 1,2-dibromobenzene	δ 7.84 (H3, d, J = 2.0 Hz), 7.66 (H5, dd, J = 2.3, 8.7 Hz), 7.14 (H6, d, J = 8.1 Hz), 7.63 ($H_{2'}$, d, J = 2.2 Hz), 7.68 (H5′, d, J = 8.1 Hz), 7.17 (H6′, dd, J = 2.0, 8.3 Hz)
2,3′,4′,5-tetra (466)	2,5-dibromoaniline, 1,2-dibromobenzene	Robertson et al. (1980) (99%)
3,3′,4,4′-tetra (466)	3,4-dibromoaniline, 1,2-dibromobenzene	Robertson et al. (1982) (99%)
2,2′,4,5,5′-penta (544)	2,4,5-tribromoaniline, 1,4-dibromobenzene	δ 7.92 (H3, s), 7.48 (H6, s), 7.31–7.63 (H3′, –4′, –6′, m) (in deuteriochloroform) (99%)
2,3′,4,4′,5-penta (544)	2,4,5-tribromoaniline, 1,2-dibromobenzene	Robertson et al. (1980) (96%)
3,3′,4,4′,5-penta (622)	3,4-dibromoaniline, 1,2,3-tribromobenzene	Robertson et al. (1982) (99%)
2,2′,3,4,4′,5′-hexa (622)	2,4,5-tribromoaniline, 1,2,3-dibromobenzene	δ 7.67 (H5, d, J = 8.3 Hz), 7.00 (H6, d, J = 8.1 Hz), 7.93 (H3, s), 7.45 (H6′, s) in deuteriochloroform) (99%)
2,3′,4,4′,5-penta (544)	2,4,5-tribromoaniline, 1,2-dibromobenzene	Robertson et al. (1980) (96%)
3,3′,4,4′,5-penta (622)	3,4-dibromoaniline, 1,2,3-tribromobenzene	Robertson et al. (1982) (99%)
2,2′,3,4,4′,5′-hexa (622)	2,4,5-tribromoaniline, 1,2,3-tribromobenzene	δ 7.67 (H5, d, J = 8.3 Hz), 7.00 (H6, d, J = 8.1 Hz), 7.93 (H3, s), 7.45 (H6′, s) in deuteriochloroform) (99%)
2,2′,3,4′,5′,6-hexa (622)		gift from S. D. Aust
2,2′,4,4′,5,5′-hexa (622)	from fireMaster	Safe et al. (1978) (99%)
2,3,3′,4,4′,5-hexa (622)	2,3,4,5-tetrabromoaniline, 1,2-dibromobenzene	Robertson et al. (1981a) (90%)
2,3,3′,4,4′,5′-hexa (622)	3,4,5-tribromoaniline, 1,2,3-tribromobenzene	δ 7.66 (H5, d, J = 8.3 Hz), 7.06 (H6, d, J = 8.3 Hz), 7.55 (H2′, –6′, s) (in deuteriochloroform) (99%)
2,3′,4,4′,5,5′-hexa (622)	2,4,5-tribromoaniline, 1,2,3-tribromobenzene	δ 7.93 (H3, s), 7.53 (H6, s), 7.58 (H2′, –6′, s) (in deuteriochloroform) (96%)
3,3′,4,4′,5,5′-hexa (622)	diazotization of 3,3′,5,5′-tetrabromobenzidine	Sundstrom et al. (1976) (98%)
2,2′,3,3′,4,4′,5-hepta (700)	gift from S. D. Aust	Moore and Aust (1978); Moore et al. (1980)
2,2′,3,4,4′,5,5′-hepta (700)	gift from S. D. Aust	Moore and Aust (1978); Moore et al. (1980)
2,2′,3,4′,5,5′,6-hepta (700)	2,4,5-tribromoaniline, 1,2,4,5-tetrabromobenzene	Robertson et al. (1981a–c) (96%)

nated benzene followed by extensive column and thin-layer chromatography as previously described (Robertson et al., 1980, 1981a, 1982). The structures of all compounds were confirmed by their proton magnetic resonance (^1H NMR) and mass spectra. The ^1H NMR spectra of all PBB congeners that have not been previously reported are summarized in Table I. FireMaster BP-6, lot 7062, was provided by the Michigan Chemical Co., St. Louis, MI. 3-Methylcholanthrene, benzo[a]pyrene, ethyl isocyanide, NADP, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co.; carbon monoxide was purchased from Matheson Chemical Co. Tritiated benzo[a]pyrene (20 Ci $mmol^{-1}$) was purchased from Amersham Corp. and purified as described (Robertson et al., 1982).

Analytical Methodology. High-resolution gas chromatography (GC) was performed with a Varian 3700 gas chromatograph equipped with a ^{63}Ni electron-capture detector. Congeneric PBBs were separated on a 50-m fused silica capillary column (0.20-mm i.d.) coated with SE-54 (Hewlett-Packard). The oven temperature was programmed to increase from 100 to 280 °C at a rate of 1.0 °C/min and was constant held at 280 °C until all PBBs eluted. The injector and detector temperatures were 240 and 330 °C, respectively. Samples (4.5 μL) were injected with an automatic sampler with splitting in the injector (10:1 split ratio, vented from 0.75 to 1.75 min). The hydrogen carrier gas was held at a constant pressure of 2.25 kg/cm^2 to give an optimized linear velocity (u) of 45.0 cm/s at 100 °C.

The structures of the PBB congeners present in fireMaster BP-6 (lot 7062) were confirmed by capillary gas chromatography–negative ion chemical ionization mass spectrometry using methane as the reagent gas. A 45-m Apiezon M fused silica capillary column (0.23-mm i.d.) deactivated with a thin film (0.025 μm) of Polysiloxane was employed to separate the PBB mixtures by using the following operating conditions: carrier gas (helium, 0.6 mL/min); splitless injection for 0.4 min followed by a 10:1 split; column temperature held at 100 °C for 0.1 min followed by temperature programming from 100 °C to 260 °C at 1.5 °C/min. The mass spectrometer operating conditions were as follows: forepressure, 0.2 torr; high pressure, 4.2×10^{-5} torr; manifold temperature 120 °C; ionizer, 0.5 mA; electron energy, –70 V; length of scan, 1.05; scan range, 30–1000 amu. Relative retention times of authentic PBB standards and the PBB congeners in fireMaster BP-6 were determined by using three internal standards: 1,2-dichloronaphthalene, 1,2,3,4-tetrachloronaphthalene, and octachloronaphthalene. The results were comparable to those reported in Table II, and the molecular ions for the PBBs in fireMaster are also noted in Table I.

Animal Treatment and Isolation of Microsomes. Immature male Wistar rats, approximately 100 g, were housed in cages and allowed free access to Purina Rat Chow No. 5002 and water. Animals were maintained on a diurnal cycle of 12 h of light and 12 h of dark. Animals were injected ip with the test compounds in 0.5 mL of corn oil at specific dose levels (150 or 60 μmol/kg) on days 1 and 3 of the experiment. Phenobarbital (400 μmol/kg) dissolved in isotonic saline and 3-methylcholanthrene (100 μmol/kg) dissolved in corn oil were administered to animals on days 3, 4, and 5. A control group was injected with corn oil on days 1 and 3. All animals were fasted for 24 h to lower liver glycogen levels before being killed by cervical dislocation on day 6.

Liver microsomes were prepared as described (Parkinson et al., 1980), after the livers were perfused with ice-cold isotonic saline via the hepatic portal vein. Protein concentrations, the reduced cytochrome P-450:carbon monoxide and ethyl isocyanide binding difference spectra, and NADPH–cytochrome c reductase, aminopyrine N-dimethylase, and aryl hydrocarbon (benzo[a]pyrene) hydroxylase (AHH) activities were determined as previously

Table II. Composition of FireMaster BP-6 As Determined by Capillary GC Using Authentic Standards and a Summary of Congeners That Induce AHH

(no.) structure	rel RT obsd[a]	rel RT calcd	rel response factors[b]	% composition of fireMaster	AHH induction activity
(018) 2,2′,5-	0.5226	0.529	1.0973	0.050	
(026) 2,3′,5-	0.5934	0.601	1.1214	0.024[e]	
(031) 2,4′,5-	0.6095	0.615	1.3660	0.015[e]	
(037) 3,4,4′-	0.7081	0.701	0.9378	0.021	active
(049) 2,2′,4,5′-	0.7228	0.719	1.0903	0.025	
(052) 2,2′,5,5′-	0.7102	0.715	1.1850	0.052	
(066) 2,3′,4,4′-	0.8202	0.805	0.9773	0.028	active
(070) 2,3′,4′,5-	0.8116	0.801	1.1527	0.017	
(077) 3,3′,4,4′-	0.9134	0.887	0.8805	0.159	active
(101) 2,2′,4,5,5′-	0.9018	0.905	1.0680	2.69	
(118) 2,3′,4,4′,5-	1.0019	0.991	0.9230	2.94	active
(126) 3,3′,4,4′,5-	1.0939	1.073	0.4493	0.079	active
(138) 2,2′,3,4,4′,5′-	1.1197	1.112	0.4156	12.3	active
(149) 2,2′,3,4′,5′,6-	1.0347	1.009	0.3960[c]	2.24	
(153) 2,2′,4,4′,5,5′-	1.0808	1.095	1.1373	53.9	
(156) 2,3,3′,4,4′,5-	1.1727	1.203	0.1037	0.980	active
(157) 2,3,3′,4,4′,5′-	1.2049	1.203	0.0971	0.526	active
(167) 2,3′,4,4′,5,5′-	1.1671	1.193	0.3039	7.95	active
(169) 3,3′,4,4′,5,5′-	1.i598	1.259	0.1021	0.294	active
(170) 2,2′,3,3′,4,4′,5-	1.3089	1.333	0.7335[d]	0.256	?[f]
(180) 2,2′,3,4,4′,5,5′-	1.2684	1.306	0.7335	6.97	
(187) 2,2′,3,4′,5,5′,6-	1.1897	1.195	0.7335	0.392	
total				91.9	

[a] Octachloronaphthalene (OCN) = 1.000. [b] 1 pg of OCN = 1.0000. [c] Calculated from average relative response factor (RRF) for all hexabromobiphenyls analyzed. [d] RRF for isomer 187 used. [e] Numbers obtained from previous work on another column. [f] The PCB analogue induces microsomal AHH.

described (Robertson et al., 1982).

RESULTS

Table I summarizes the reactants used to synthesize the PBB congeners. With the exception of the three PBBs donated by Dr. S. D. Aust, the purity of each PBB congener was determined by GLC. Unless previously reported, the ^{1}H NMR data for the synthetic PBBs are also summarized in Table I. The levels of 22 PBBs in fireMaster BP-6 are summarized in Table II, along with the retention time and response factor of each PBB congener (expressed relative to those of octachloronaphthalene). The PBB congeners listed in Table II are numbered according to the scheme proposed by Ballschmiter and Zell (1980) for PCBs. The GC analysis accounted for more than 91% of the PBB component in fireMaster BP-6.

Many of the PBBs identified in fireMaster (Table II) have been previously examined for their ability to induce rat liver microsomal drug-metabolizing enzymes (Dannan et al., 1978, 1983; Parkinson et al., 1983; Robertson et al., 1980, 1981a, 1982). Those synthetic PBBs that had not been examined prior to this study were administered to immature male Wistar rats, and these PBBs were compared to phenobarbital and 3-methylcholanthrene as inducers of liver microsomal cytochrome P-450. Three congeners, namely, 2,2′,5,5′-tetra-, 2,2′,4,5,5′-penta-, and 2,2′,4,4′,5,5′-hexachlorobiphenyl, were apparently phenobarbital-type inducers of cytochrome P-450, aminopyrine *N*-dimethylase, and NADPH–cytochrome *c* reductase. At the dose tested, neither congener induced aryl hydrocarbon hydroxylase. Three other congeners, namely, 2,3′,4,4′-tetra-, 2,2′,3,4,4′,5′-hexa-, and 2,3′,4,4′,5,5′-hexabromobiphenyl, exhibited both phenobarbital- and 3-methylcholanthrene-type inducing characteristics. The induction of aryl hydrocarbon hydroxylase by these mixed-type inducers was accompanied by peak shifts in the absorbance maxima of the adducts between reduced cytochrome P-450 and either carbon monoxide or ethyl isocyanide. A seventh compound, 2,3,3′,4,4′,5-hexabromobiphenyl, was a 3-methylcholanthrene-type inducer of cytochrome P-450 (P-448) and aryl hydrocarbon hydroxylase. At the dose tested, this congener did not significantly increase the activity of either aminopyrine *N*-demethylase or NADPH–cytochrome *c* reductase. The induction patterns for all these compounds are summarized in Table II.

DISCUSSION

The toxicological evaluation of fireMaster BP-6 and the PBB mixtures that persist in environmental samples and humans has been hindered by the difficulties encountered in synthesizing authentic standards due to the lack of synthetic precursors. This has not been the case with PCBs (Mullin et al., 1981), and the synthesis of all 209 isomers and congeners has recently been accomplished in our laboratory. Our approach to the toxicological evaluation of fireMaster has been (a) developing structure–activity rules for predicting the toxicity of individual PBBs (and PCBs), (b) synthesizing the predicted toxic compounds, and (c) determining the presence (or absence) of these PBB congeners in various mixtures by comparative high-resolution GC and GC–MS analysis.

The identification of the potentially toxic PBB congeners has been facilitated by studies on PCBs, PCDDs, and related halogenated aryl hydrocarbons, which revealed that there is an excellent correlation between those compound that induce microsomal aryl hydrocarbon hydroxylase (AHH) and those that are toxic (Safe et al., 1982; Poland and Knutson, 1982). This correlation is not merely fortuitous but rather exists because both aryl hydrocarbon hydroxylase induction and toxicity appear to be mediated through a common cytosolic receptor (Poland and Knutson, 1982). We previously reported that at least five PBBs that are approximate isostereomers of 2,3,7,8-TCDD (the most toxic halogenated aryl hydrocarbon) are potent aryl

hydrocarbon hydroxylase inducers (Robertson et al., 1982). Moreover, on the basis of previous studies with PBBs and PCBs, the mono- and some di-ortho derivatives of these five PBBs should also exhibit some toxicity (Parkinson et al., 1980, 1981, 1983; Robertson et al., 1980, 1981a, 1982, 1983; Parkinson et al., 1980, 1981, 1983).

Like other toxic halogenated aromatics there is evidence that suggests that the toxicities of fireMaster PB-6 and individual PBB congeners are associated with those specific compounds that induce AHH. Chromatographic separation of fireMaster BP-6 by alumina or Florisil column chromatography gives a polar fraction and nonpolar fraction that differ in their biologic and toxic effects. The polar PBB fraction is a potent inducer of microsomal AHH and causes hyperkeratosis in the rabbit ear test whereas the nonpolar fraction is relatively inactive (Needham et al., 1982; Robertson et al., 1981c). Photolytic degradation of fireMaster BP-6 or 2,2′,4,4′,5,5′-hexabromobiphenyl results in material that exhibits increased AHH-inducing activity and toxicity, and this is associated with the photolytic formation of specific PBB congeners that induce AHH (Robertson et al., 1981a–c; Patterson et al., 1981). The correlation between AHH induction and toxic potencies is also supported by results reported for several individual PBBs; the coplanar PBB congeners, 3,3′,4,4′-tetra-, 3,3′,4,4,5′-penta-, and 3,3′,4,4′,5,5′-hexabromobiphenyl, induce microsomal AHH or the associated cytochrome P-450 isozymes (Robertson et al., 1982; Dannan et al., 1983; Parkinson et al., 1983) and elicit several toxic effects that are also included for 2,3,7,8-TCDD and related isostereomers. These effects include cleft palate in genetically inbred responsive mice (Poland and Glover, 1980), rabbit ear hyperkeratosis (Orti et al., 1983), thymic atrophy and body weight loss in rats (Robertson et al., 1983; Parkinson et al., 1983; Render et al., 1982; Akoso et al., 1982), liver toxicity (Akoso et al., 1982; Render et al., 1982; Robertson et al., 1983), and tumor-promoting activity (Poland et al., 1982). Several studies show that mono-*o*-bromo derivatives of the copolanar PBBs also induce AHH and elicit some of the toxic effects reported for the more active coplanar compounds (Parkinson et al., 1983; Akoso et al., 1982; Dannan et al., 1983; Robertson et al., 1980, 1982). This study adds three new synthetic compounds to the list of PBB congeners that induce AHH, namely, 2,3′,4,4′-tetra-, 2,2′,3,4,4′,5-hexa-, and 2,3,3′,4,4′,5-hexabromobiphenyl. Moreover, we also report the synthesis and AHH-inducing activity of 2,3′,4,4′,5,5′-hexabromobiphenyl, a toxic PBB congener that has previously been isolated from the crude commercial PBB mixture (Dannan et al., 1978). The monooxygenase enzyme inducing activities of three additional compounds present in fireMaster, 2,2′,5,5′-tetra-, 2,2′,4,5,5′-penta-, and 2,2′,4,4′,5,5′-hexabromobiphenyl, resemble PB in their mode of induction.

On the basis of previous toxicological studies with individual PBB isomers and congeners or their chlorinated analogues, 3,3′,4,4′-tetra-, 3,3′,4,4′,5-penta-, and 3,3′,4,4′,5,5′-hexabromobiphenyls (coded in Figure 1 and Table II at 077, 126, and 169, respectively) are the most toxic congeners identified in fireMaster BP-6. Of all the individual PBBs examined, these three coplanar congeners are the most potent inducers of aryl hydrocarbon hydroxylase (Robertson et al., 1982, 1983). Although these three coplanar PBBs have not been isolated from the fireMaster mixture, their presence in fireMaster is supported by cochromatography with authentic standards in two high-resolution capillary GC systems, by GC–MS and by an independent analytical study using high-pressure

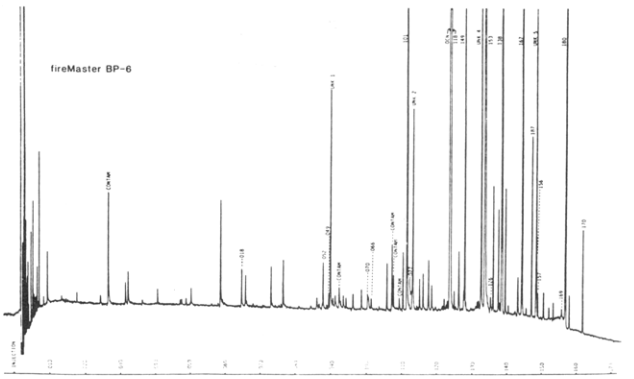

Figure 1. High-resolution capillary gas chromatogram of the commercial polybrominated biphenyl mixture, fireMaster BP-6, lot 7062. (CONTAM, solvent contaminants; OCN, octachloronaphthalene included as an internal standard; UNK, unknown, unidentified fireMaster components causing at least half full-scale pen deflection).

liquid chromatography and spectroscopic confirmation of structure (Orti et al., 1983). Furthermore, it is apparent from the retention time data (Table II) that within each isomeric series the rate of elution of each PBB was highly dependent on its structure. For example, the retention times of isomeric PBBs tends to be inversely related to the degree of ortho bromination. In addition, as previously noted for PCB congeners (Parkinson et al., 1980, 1981), ortho halogens buttressed by a meta and para halogen (i.e., the 2,3,4-substitution pattern) tend to exhibit more coplanar character. This is clearly illustrated by the comparison of PBBs 138 and 153, each possessing two ortho, two meta, and two para bromines yet differing greatly in their time of elution. In an attempt to relate these various factors, the data for the hexabromobiphenyls were subjected to linear model analysis and the followng mathematical relationship was derived [a refinement of the approach given by Sweetman and Boettner (1982)], correlation the relative retention times (RRT) with the number of ortho (*o*), buttressed ortho (*o*b), meta (*m*), and para (*p*) bromines:

$$\mathrm{RRT} = 0.135 + 0.104o + 0.130o\mathrm{b} + 0.186m + 0.190p$$

Table II illustrates the excellent correlation ($r^2 = 0.997$) between the observed and calculated retention times for the isomeric hexabromobiphenyls. These results support the peak structure assignments and provide further evidence for the presence of 3,3′,4,4′-tetra-, 3,3′,4,4′,5-penta-, and 3,3′,4,4′,5,5′-hexabromobiphenyl in fireMaster BP-6.

Table II summarizes the concentrations and identities of the 22 PBB congeners identified in fireMaster BP-6 and their activity as AHH inducers (Dannan et al., 1978, 1983; Parkinson et al., 1983; Robertson et al., 1980, 1981a, 1982). A recent paper (Orti et al., 1983) has confirmed the presence of many of these compounds and four additional PBB congeners not identified in this study, namely, 2,2′,3,5′,6-penta-, 2,2′,3,3′,4,5,5′-hepta-, 2,3,3′,4,4′,5-hepta-, and 2,2′,3,3′,4,5,6′-heptabromobiphenyl. Orti et al. (1983) also confirm the presence of the highly toxic 3,3′,4,4′-tetra-, 3,3′,4,4′,5-penta-, and 3,3′,4,4′,5,5′-hexabromobiphenyls in fireMaster FF-1 (lot FH 7042). The detection of these toxins in two different commercial PBB lots suggests that these compounds are minor components of fireMaster; however, their relative concentrations in different preparations may be variable.

Thus, it is apparent that 3,3′,4,4′-tetra-, 3,3′,4,4′,5-penta-, and 3,3′,4,4′,5,5′-hexabromobiphenyls represent at least some of the previously unidentified toxic components that have long been suspected to exist in fireMaster. It is

significant in this regard that, on the basis of their known elution profiles during Florisil and alumina column chromatography, the presence of these coplanar PBBs in fireMaster provides a possible explanation for the toxic potencies of various chromatographic fractions prepared from fireMaster BP-6 (Kimbrough et al., 1977; Robertson et al., 1981c).

Clearly, future work must focus on the identification, synthesis, and toxicologic evaluation of all the components of fireMaster and their preferential bioconcentration in the exposed human populations. This will then permit a more rational assessment of the potential long-term health effects of PBBs.

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Registry No. AHH, 9037-52-9; fireMaster BP-6, 59536-65-1; 2,2′,5-tribromobiphenyl, 59080-34-1; 2,3′,5-tribromobiphenyl, 59080-35-2; 2,4′,5-tribromobiphenyl, 59080-36-3; 3,4,4′-tribromobiphenyl, 6683-35-8; 2,2′,4,5′-tetrabromobiphenyl, 60044-24-8; 2,2′,5,5′-tetrabromobiphenyl, 59080-37-4; 2,3′,4,4′-tetrabromobiphenyl, 84303-45-7; 2,3′,4′,5-tetrabromobiphenyl, 59080-38-5; 3,3′,4,4′-tetrabromobiphenyl, 77102-82-0; 2,2′,4,5,5′-pentabromobiphenyl, 67888-96-4; 2,3′,4,4′,5-pentabromobiphenyl, 67888-97-5; 3,3′,4,4′,5-pentabromobiphenyl, 84303-46-8; 2,2′,3,4,4′,5′-hexabromobiphenyl, 67888-98-6; 2,2′,3,4′,5′,6-hexabromobiphenyl, 69278-59-7; 2,2′,4,4′,5,5′-hexabromobiphenyl, 59080-40-9; 2,3,3′,4,4′,5-hexabromobiphenyl, 77607-09-1; 2,3,3′,4,4′,5′-hexabromobiphenyl, 84303-47-9; 2,3′,4,4′,5,5′-hexabromobiphenyl, 67888-99-7; 3,3′,4,4′,5,5′-hexabromobiphenyl, 60044-26-0; 2,2′,3,3′,4,4′,5-heptabromobiphenyl, 69278-60-0; 2,2′,3,4,4′,5,5′-heptabromobiphenyl, 67733-52-2; 2,2′,3,4′,5,5′,6-heptabromobiphenyl, 84303-49-1; cytochrome P-450, 9035-51-2; aminopyrine *N*-demethylase, 9037-69-8; NADPH–cytochrome *c* reductase, 9023-03-4.

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