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POTENT INDUCTION OF RAT LIVER MICROSOMAL, DRUG-METABOLIZING ENZYMES BY 2,3,3',4,4',5-HEXABROMOBIPHENYL, A COMPONENT OF FIREMASTER

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SUMMARY

The multistep synthesis and purification of 2,3,3',4,4',5-hexabromobiphenyl (HBBp) is described. Capillary gas chromatography revealed that HBBp comprises 0.05% of the industrial polybrominated biphenyl (PBB) mixture, fireMaster BP-6 (lot 7062). When administered to immature male Wistar rats, HBBp caused a dose-dependent increase in (a) the activity of benzo[*a*]pyrene (B[*a*]P) hydroxylase (AHH) and 4-chlorobiphenyl (4-CBP) hydroxylase and (b) the concentration of cytochrome *P*-450. Sodium dodecyl sulfate (SDS)-gel electrophoresis indicated that these increases in cytochrome *P*-450 and cytochrome *P*-450-dependent monooxygenase activities were accompanied by a dose-dependent intensification of a protein of relative molecular weight (Mr) 55 000 which comigrated with the major 3-methylcholanthrene(MC)-inducible form of cytochrome *P*-450 (i.e., cytochrome *P*-448). Like MC, but in contrast to phenobarbitone (PB), HBBp competitively displaced 2,3,7,8-[^{3}H]tetrachlorodibenzo-*p*-dioxin ([^{3}H]-TCDD) from the cytosolic protein thought to be the receptor for cytochrome *P*-448 induction. The results indicate that HBBp is a potent inducer of cytochrome *P*-448 and as such is the third MC-type inducer identified in fireMaster BP-6.

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Abbreviations: AHH, aryl hydrocarbon hydroxylase; B[*a*]P, benzo[*a*]pyrene; CO, carbon monoxide; 4-CBP, 4-chlorobiphenyl; DMAP, 4-dimethylaminoantipyrine; EIC, ethylisocyanide; GC, gas chromatography; HBBp, 2,3,3',4,4',5-hexabromobiphenyl; HEDG, Hepes-EDTA-dithiothreitol-glycerol; MC, 3-methylcholanthrene; Mr, relative molecular weight; MS, mass spectrometry; PB, phenobarbitone; PBB, polybrominated biphenyl; PCB, polychlorinated biphenyl; PMR, proton magnetic resonance; SDS, sodium dodecyl sulfate; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TLC, thin-layer chromatography.

INTRODUCTION

The commercial PBB mixture, fireMaster BP-6 is a mixed (PB + MC)-type inducer of both cytochrome *P*-450- and *P*-448-dependent monooxygenases [1]. The two major components of fireMaster, namely 2,2′,4,4′,-5,5′-hexabromobiphenyl and 2,2′,3,4,4′,5,5′-heptabromobiphenyl, are strictly PB-type inducers of cytochrome *P*-450 [2,3] and ostensibly account for the majority of the PB-type character of the commercial mixture. 2,3′,4,4′,5-Pentabromobiphenyl [4] and 2′,3,4,4′,5,5′-hexabromobiphenyl [5] are reported to be mixed-type inducers. However the potency with which these two congeners induce cytochrome *P*-448 and other evidence [1] clearly indicate that additional components in fireMaster contribute to its MC-type character. We are currently attempting to identify such components which induce cytochrome *P*-448-dependent monooxygenases, specifically B[*a*]P hydroxylase (AHH), and report here on the synthesis, quantitation in fireMaster BP-6 and mode of induction of HBBp.

MATERIALS AND METHODS

Synthesis

HBBp and 2,3,3′,4,4′,5,6-heptabromobiphenyl were synthesized from 2,6-dibromo-4-nitroaniline and *o*-dibromobenzene (Aldrich Chemical Company) according to the synthetic scheme outlined in Fig. 1. The identity of the synthetic products was confirmed by mass spectrometry (MS) and 60 MHz proton magnetic resonance (PMR) spectroscopy. Chemical purities were

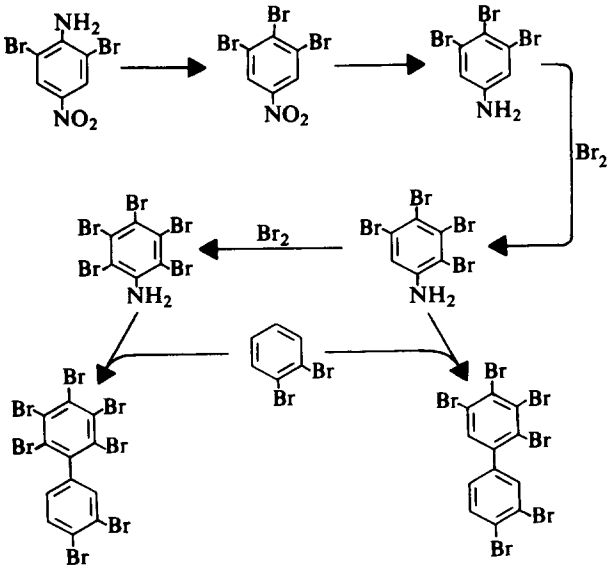

Fig. 1. Synthetic scheme for 2,3,3′,4,4′,5-hexabromobiphenyl (HBBp) and 2,3,3′,4,4′,5,6-heptabromobiphenyl (Impurity).

determined by gas-liquid chromatography using a Hewlett-Packard model 5710A gas chromatograph (1.2 m × 4 mm glass column packed with 1.5% OV 17 and 2% OV 210 on Gas Chrom Q 100/120 mesh) equipped with a flame ionization detector. The chromatograph was equipped with a Hewlett-Packard Model 3380S integrator recorder.

3,4,5-Tribromonitrobenzene was synthesized from 2,6-dibromo-4-nitroaniline in approx. 95% yield by the sulfuric acid diazotization method of Van Roosmalen [6] and its structure confirmed by MS, molecular ion at 357 *m/e*, and PMR, singlet at 8.42 ppm in $CDCl_3$.

3,4,5-Tribromoaniline was synthesized in approx. 70% yield by iron-glacial acetic acid reduction of 3,4,5-tribromonitrobenzene. The aniline was recrystallized to 95% purity from ethanol/water and its structure confirmed by MS, molecular ion at 327 *m/e*, and PMR, N singlet at 3.76 ppm and 2,6 singlet at 6.90 ppm in $CDCl_3$.

2,3,4,5-Tetrabromoaniline was synthesized from 3,4,5-tribromoaniline by careful bromination (Br_2, $CHCl_3$) in the presence of iodine and iron metal catalyst. The reaction was monitored by gas chromatography (GC) to prevent over-bromination. The product was recrystallized to 90% purity (10% being the over-bromination product, 2,3,4,5,6-pentabromoaniline) from methanol/water.

2,3,4,5,6-Pentabromoaniline was synthesized as above except that excess Br_2 was used. The product was recrystallized to greater than 99% purity from ethanol/water and its structure confirmed by MS, molecular ion at *m/e* 483.

HBBp was synthesized by the Cadogan coupling [7] of 2,3,4,5-tetrabromoaniline and *o*-dibromobenzene in the presence of amyl nitrite (Pfaltz and Bauer). The crude product was purified by chromatography on Florisil, repeated thin-layer chromatography (TLC), as described for the polychlorinated biphenyl (PCB) congeners [8], and high pressure liquid chromatography (Beckman Isocratic Liquid Chromatograph, Model 330, equipped with an Altex Ultrasil ODS 10 μm, 10 mm ID semi-preparative column, eluent: methanol). The product was found to be 90% pure (the 10% impurity being the 2,3,3′,4,4′,5,6-heptabromobiphenyl). The structure of HBBp was confirmed by MS, molecular ion at *m/e* 622, and PMR; H_6: 7.53 ppm (s), $H_{2'}$: 7.58 ppm (d, J = 1.9 Hz), $H_{5'}$: 7.68 ppm (d, J = 7.8 Hz), $H_{6'}$: 7.12 ppm (dd, J = 1.9, 7.8 Hz) in $CDCl_3$.

2,3,3′,4,4′,5,6-Heptabromobiphenyl (Impurity) was synthesized by the Cadogan coupling of 2,3,4,5,6-pentabromoaniline and *o*-dibromobenzene and purified to >96% by Florisil and TLC as described above. The structure of the heptabromobiphenyl was confirmed by MS, molecular ion at *m/e* 700, and PMR; $H_{2'}$: 7.70 ppm (d, J = 1.9 Hz), $H_{5'}$: 7.92 ppm (d, J = 7.8 Hz), $H_{6'}$: 7.24 ppm (dd, J = 1.9, 7.8 Hz) in $(CD_3)_2CO$.

Biochemicals

Cytochrome *c* (horse heart, type III), $NADP^+$, NADPH, D-glucose 6-phosphate, D-glucose-6-phosphate dehydrogenase (Baker's yeast), MC,

B[*a*]P and ethylisocyanide (EIC) were purchased from Sigma Chemical Co.; 4-dimethylaminoantipyrine (DMAP) from Aldrich Chemical Co; carbon monoxide (CO) (research purity) from Matheson and sodium phenobarbitone (PB) from the Ontario Veterinary College, Guelph. [^{3}H]B[*a*]P was obtained from New England Nuclear Co. and purified by TLC using hexane as eluent. [^{3}H]TCDD (spec. act., 50—52 Ci/mmol) was purchased from KOR Isotopes (Cambridge, MA). The radiolabelled TCDD contained approx. 80% of the TCDD congener, as judged by the manufacturer and confirmed in our laboratory by GC.

Animal treatment and isolation of microsomes

One-month-old male Wistar rats, average weight 100 g, were housed in wire cages and allowed free access to Purina Certified Rodent Chow #5002 and water. HBBp was dissolved in corn oil and doses of 1.9, 3.8, 7.5, 15 and 30 μmol · kg^{-1} were administered at each dose level by intraperitoneal injection on days 1 and 3. The animals were killed by cervical dislocation on day 6. 2,3,3′,4,4′,5,6-Heptabromobiphenyl was administered in corn oil at 3.0 μmol · kg^{-1} as above. PB (400 μmol · kg^{-1}), dissolved in isotonic saline and MC (100 μmol · kg^{-1}), dissolved in corn oil, were administered individually as well as coadministered to animals on days 1, 2 and 3 and the animals killed on day 4. Animals injected with corn oil (5 ml · kg^{-1}) served as controls which, along with PB-, MC- and PB + MC-treated rats, were included in each experiment and no significant variation was observed from week to week. All animals were fasted over the last 24 h to lower liver glycogen levels.

The rat livers were perfused via the hepatic portal vein with ice-cold isotonic saline supplemented with EDTA (0.1 mM). The blanched livers were transferred to preweighed, ice-cold solutions of sucrose/EDTA (0.25 M/0.1 mM) and the liver weights determined. The microsomal fraction was collected as a 100 000 × *g* pellet by further centrifugation of a 10 000 × *g* supernatant from the liver homogenate.

Assays

In all assays the final concentration of microsomal protein was 1.0 mg · ml^{-1} as determined by the method of Lowry et al. [9]. The cytochrome *P*-450 content was determined by the method of Omura and Sato [10], from the CO-difference spectrum of dithionite-reduced microsomes using an extinction coefficient of 91 cm^{-1} mM^{-1} between A_{max} and A_{490}. The EIC-difference spectrum was determined in a similar manner to the CO-difference spectrum except that EIC was added to the sample cuvette (final conc., 4.5 mM) instead of CO. The concentration of cytochrome b_5 was determined from the difference spectrum between NADH-reduced microsomes and oxidized microsomes [11] using the corrected extinction coefficient of 185 cm^{-1} mM^{-1} [10]. All spectra were recorded on a Cary 118C spectrophotometer with a repetitive scan accessory. Holmium oxide was used to calibrate all spectra.

The rate of oxidative N-demethylation of DMAP was measured by quantifying the production of formaldehyde as described [12]. The formaldehyde, trapped as the semicarbazone, was developed in double-strength Nash reagent [13]. The rate of B[*a*]P hydroxylation was measured by the radiometric assay of DePierre et al. [14] as improved by Nesnow et al. [15], by quantifying the base-soluble metabolites following hexane-extraction of the unreacted B[*a*]P. The activity of 4-CBP hydroxylase was measured by the differential extraction procedure used for B[*a*]P hydroxylase as previously reported by Parkinson et al. [16]. The activity of NADPH-cytochrome *P*-450 reductase was measured by the rate of reduction of cytochrome *c* [17]. To prolong the linearity of the cytochrome *c* reduction, samples with high NADPH-cytochrome *P*-450 reductase activity were diluted to a final microsomal protein concentration of 0.2 mg · ml^{-1}.

The competitive binding of TCDD with HBBp for the cytosolic receptor protein was characterized by sucrose density gradient centrifugation analysis following dextran-charcoal treatment as described [18]. All samples contained 1 ml (5—6 mg protein) of hepatic cytosol (postmicrosomal supernatant prepared in Hepes-EDTA-dithiothreitol-glycerol (HEDG) buffer, pH 7.6) and were incubated for 1 h at 0—5°C with [^{3}H]TCDD (10 nM) added in 10 μl DMSO. Various concentrations of competitors (HBBp, MC or PB) were added in 10 μl DMSO while controls received DMSO alone. After incubation, the unbound and loosely bound [^{3}H]TCDD and/or the competitor were removed by agitating the cytosol with a dextran-charcoal (10 mg charcoal/mg dextran) pellet. Following a 15-min incubation at 0—5°C, the dextran-charcoal was removed by centrifugation at 4000 × *g* for 15 min. An aliquot (300-μl) of the resulting supernatant was layered on a sucrose gradient (5—20% sucrose in HEDG buffer) and centrifuged in a Beckman SW 50·1 rotor at 48 000 rev./min for 16 h at 2°C. After centrifugation, 40 fractions of 120 μl were collected and the radioactivity in each sample determined.

Microsomal proteins (10 μg) were analyzed by discontinuous SDS-polyacrylamide slab gel electrophoresis essentially as described by Laemmli [19]. The stacking and separating gels (0.5 × 15 cm) contained 5% and 7.5% acrylamide, respectively. Minimum molecular weights were estimated by reference to the following proteins obtained from Boehringer Mannheim: trypsin inhibitor (21 000), carbonic anhydrase (31 000), α-amylase (45 000), 3-phosphoglycerate kinase (47 000) and bovine serum albumin (68 000). Proteins were stained with Coomassie Blue.

The statistical significance between sample means of control and treated groups for each parameter studied was analyzed by Dunnett's method for multiple comparisons with a control [20].

RESULTS

The effects of PB, MC and their coadministration (PB + MC)

PB treatment resulted in a proliferation of the endoplasmic reticulum

(as indicated by an increase in the mg microsomal protein · g liver^{-1}) in addition to a general increase in the liver to body weight ratio. Both PB and MC enhanced the concentration of cytochromes b_5 and *P*-450. The CO-difference spectrum of reduced PB-induced microsomes displayed an absorption maximum at 450 nm. The corresponding EIC-difference spectrum exhibited two major peaks at 428 and 455 nm with a 455 : 428 nm peak height ratio of about 0.7. The absorption maximum of the reduced MC-induced microsome/CO complex was shifted to 448 nm. Both absorption maxima of the reduced MC-induced microsome/EIC complex were shifted: from 428 to 429.5 nm and from 455 to 452 nm. The 452/429.5 nm peak height ratio increased to 1.8.

PB induction resulted in an increase in the concentration of cytochrome *P*-450 which was accompanied by an increase in the activity of DMAP *N*-demethylase (×2.2), B[*a*]P hydroxylase (×3.1) and NADPH-cytochrome *c* reductase (×2.5). In the case of MC, no increase in the activity of DMAP *N*-demethylase or NADPH-cytochrome *c* reductase was observed but the increase in the concentration of cytochrome *P*-448 was accompanied by a large increase in B[*a*]P hydroxylase activity (×15.4).

In contrast to other enzymic activities, PB treatment failed to significantly increase the activity of 4-CBP hydroxylase whereas this enzymic activity was increased 12.3-fold following MC treatment.

In all cases the coadministration of PB and MC resulted in increased enzymic activity and cytochrome concentration which were greater than the corresponding increases evoked by either PB or MC alone while the qualitative spectral characteristics displayed by PB + MC-induced microsomes were intermediate between PB- and MC-induced microsomes.

Mode of induction by HBBp and its impurity

Pretreatment of rats with HBBp resulted in a dose-dependent increase in the concentration of cytochrome *P*-450 and in the activities of B[*a*]P hydroxylase and 4-CBP hydroxylase. Both these monooxygenase activities were significantly increased over controls ($\alpha = 0.01$) at all doses of HBBp. The dose of HBBp effecting half-maximal induction (ED_{50}) of 4-CBP hydroxylase and B[*a*]P hydroxylase was 6.3 and 12 μmol · kg^{-1}, respectively.

Although statistically significant increases in DMAP *N*-demethylase and NADPH-cytochrome *c* reductase activity were observed following HBBp treatment, the magnitude of these increases was much less than that observed following PB treatment. With increasing doses of HBBp, the maximum wavelength (λ_{max}) of the CO-difference spectrum displayed an increasing hypsochromic shift from 450 nm to 448.9 nm. Similarly, a hypsochromic shift in the 455 nm peak and a bathochromic shift in the 428 nm peak of the EIC-difference spectrum were observed. In addition the 455 : 428 nm peak height ratio of the EIC-difference spectrum increased with increasing doses of HBBp.

The major impurity in HBBp, namely 2,3,3',4,4',5,6-heptabromobiphenyl, failed to significantly increase any parameter studied at a dose of 6 μmol ·

TABLE I

THE EFFECTS OF HBBp AND 2,3,3',4,4',5,6-HEPTABROMOBIPHENYL (IMPURITY) AS HEPATIC ENZYME INDUCERS IN MALE WISTAR RATS

Values are mean ± S.D. *Significantly different from Corn Oil Control, $\alpha = 0.01$. ND, not done

		% Liver wt. of body wt.	mg Protein g liver^{-1}	DMAP *N*-Demethylase nmol HCHO formed (mg protein^{-1} · min^{-1})	NADPH-Cytochrome *c* reductase (nmol mg protein^{-1} · min^{-1})	B[*a*]P Hydroxylase pmol B[*a*]P metabolized (mg protein^{-1} · min^{-1})	4-CBP Hydroxylase pmol 4-CBP metabolized (mg protein^{-1} · min^{-1})	Cytochrome b_5 (nmol · mg protein^{-1})	Cytochrome *P*-450 (nmol · mg protein^{-1}) (peak maximum)	EIC-Difference spectrum	
										Peak maxima (nm)	Peak height ratio (455 : 428 nm)
Corn Oil	$n = 10$	4.20 ± 0.36	33.4 ± 5.0	3.90 ± 0.26	68.0 ± 9.3	184 ± 20	109 ± 15	0.205 ± 0.016	0.665 ± 0.065 (450.0)	428.0, 455.0	0.51 ± 0.03
PB	$n = 10$	5.75 ± 0.66*	47.1 ± 2.4*	8.72 ± 0.80*	170 ± 12	572 ± 58*	121 ± 8	0.273 ± 0.024*	1.57 ± 0.13* (450.0)	428.0, 455.0	0.67 ± 0.08*
MC	$n = 10$	4.78 ± 0.38*	39.1 ± 3.1*	3.95 ± 0.41	72 ± 11	2840 ± 120*	1340 ± 70*	0.259 ± 0.011*	1.45 ± 0.08* (448.0)	429.5, 452.0	1.8 ± 0.1*
PB + MC	$n = 10$	6.31 ± 0.61*	49.1 ± 3.4*	9.00 ± 0.72*	178 ± 21*	2880 ± 120*	1410 ± 80*	0.293 ± 0.024*	2.24 ± 0.26* (448.5)	428.5, 452.0	1.1 ± 0.2*
HBBp 3.75 (μmol kg^{-1})	$n = 4$	4.40 ± 0.13	41.2 ± 4.6*	5.20 ± 0.60*	71.7 ± 6.1	408 ± 57*	445 ± 54*	0.253 ± 0.011*	0.64 ± 0.02 (450.0)	428.0, 454.8	0.54 ± 0.02
HBBp 7.5 (μmol kg^{-1})	$n = 4$	4.18 ± 0.50	41.8 ± 2.4*	5.89 ± 0.60*	70.9 ± 3.6	916 ± 190*	727 ± 114*	0.291 ± 0.012*	0.75 ± 0.06 (449.8)	428.2, 454.3	0.70 ± 0.09*
HBBp 15 (μmol kg^{-1})	$n = 4$	4.33 ± 0.19	39.8 ± 2.9*	5.06 ± 0.34*	94.6 ± 5.1*	1754 ± 233*	1030 ± 130*	0.320 ± 0.04*	0.91 ± 0.10* (449.5)	428.5, 453.8	0.83 ± 0.04*
HBBp 30 (μmol kg^{-1})	$n = 2$	4.48 ± 0.11	44.0 ± 0*	5.48 ± 0.18*	113 ± 9*	2039 ± 38*	1214 ± 41*	0.330 ± 0.014*	0.95 ± 0.07 (449.1)	428.6, 453.3	0.94 ± 0.09*
HBBp 60 (μmol kg^{-1})	$n = 1$	4.22	45.6	5.15	123	2115	1282	0.340	1.23 (448.9)	428.8, 453.0	1.14
2,3,3',4,4',5,6-Heptabromobiphenyl (6 μmol kg^{-1})	$n = 2$	4.41 ± 0.64	34.0 ± 1.6	4.01 ± 0.50	68.3 ± 3.8	179 ± 5	ND	0.237 ± 0.006	0.688 ± 0.009 (450.0)	428.0, 455.0	0.53 ± 0.01

kg^{-1}, i.e., at a dose 10X that present in the dose of HBBp causing half-maximal induction of 4-CBP hydroxylase.

Electrophoresis of microsomes from control, PB, MC, PB + MC, HBBp and 2,3,3',4,4',5,6-heptabromobiphenyl-induced rats

SDS-gel electrophoresis of crude microsomes revealed that coadministration of PB with MC caused an intensification of four protein-staining bands, arbitrarily designated A—D, with minimum Mr of 47 000, 50 000, 53 000 and 55 000, respectively (Fig. 2). Of these four polypeptides, C and, to a lesser extent, B were intensified following PB treatment while D and, to a lesser extent, A were intensified following MC treatment. HBBp treatment (Fig. 3) resulted in a dose-dependent intensification of the major MC-inducible polypeptide D. The impurity, 2,3,3',4,4',5,6-heptabromobiphenyl, failed to intensify any protein-staining band above control.

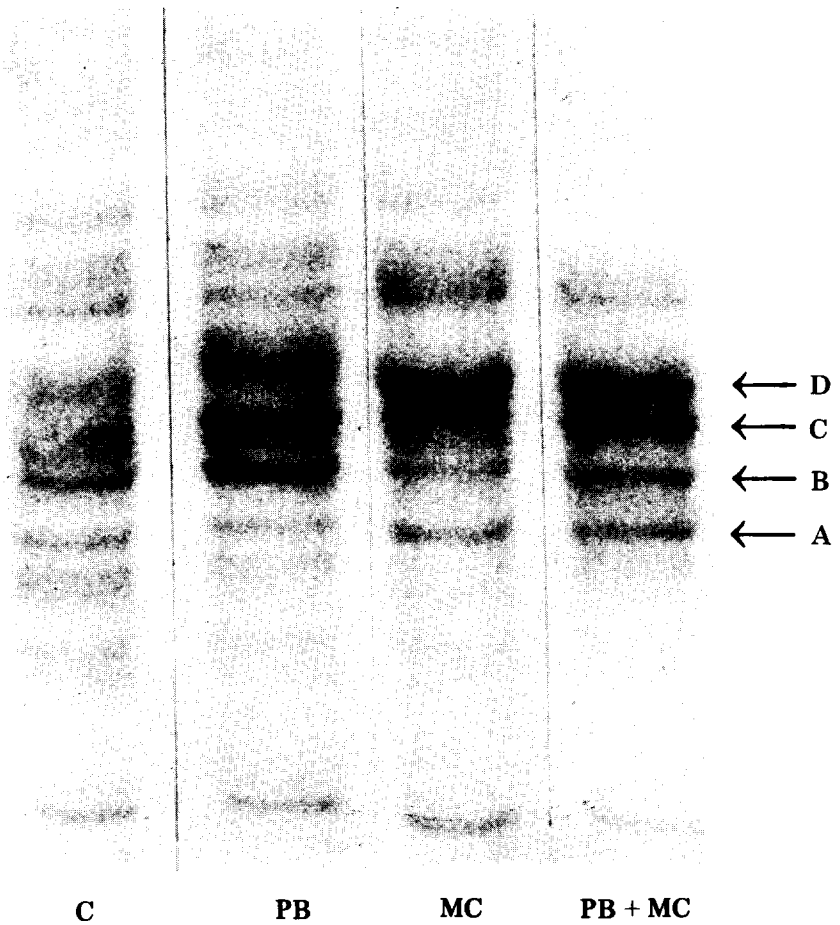

Fig. 2. SDS-polyacrylamide slab gel electrophoresis of liver microsomes from corn oil-, PB-, MC- and PB + MC-treated rats.

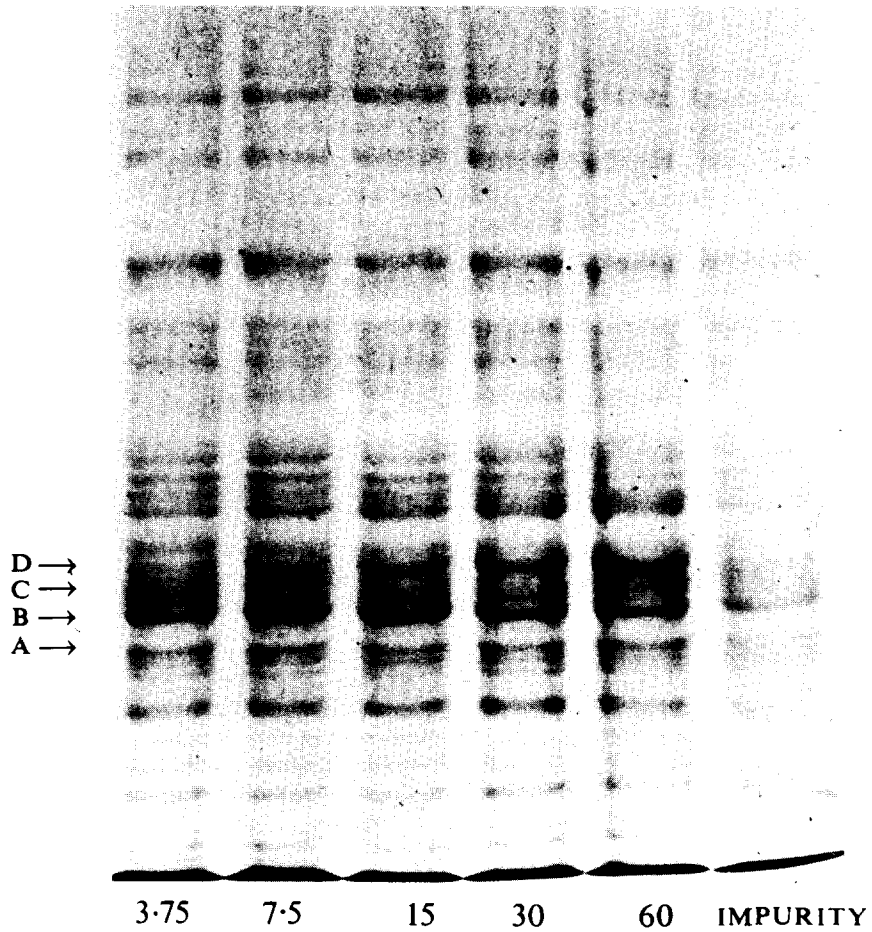

Fig. 3. SDS-polyacrylamide slab gel electrophoresis of liver microsomes from 2,3,3',4,4',5-hexabromobiphenyl (HBBp) and 2,3,3',4,4',5,6-heptabromobiphenyl (Impurity)-treated rats.

Ah receptor binding

Incubation of hepatic cytosol with 10 nM [^{3}H]TCDD for 1 h at 5°C produced a specific binding peak detectable by sucrose density gradient centrifugation analysis. The results of competitive binding between 10 nM [^{3}H]TCDD and varying concentrations of PB, MC and HBBp are shown in Fig. 4. The concentration of MC effective in displacing 50% of the specifically-bound TCDD (EC_{50}) was approx. 4.9 nM as previously reported [18]. In contrast to MC, PB failed to displace TCDD from the Ah receptor even at a concentration of 10 mM (i.e., 10^6 times the concentration of [^{3}H]TCDD). Like MC, HBBp bound to the Ah receptor but for solubility reasons the EC_{50} for this competitor could not be determined.

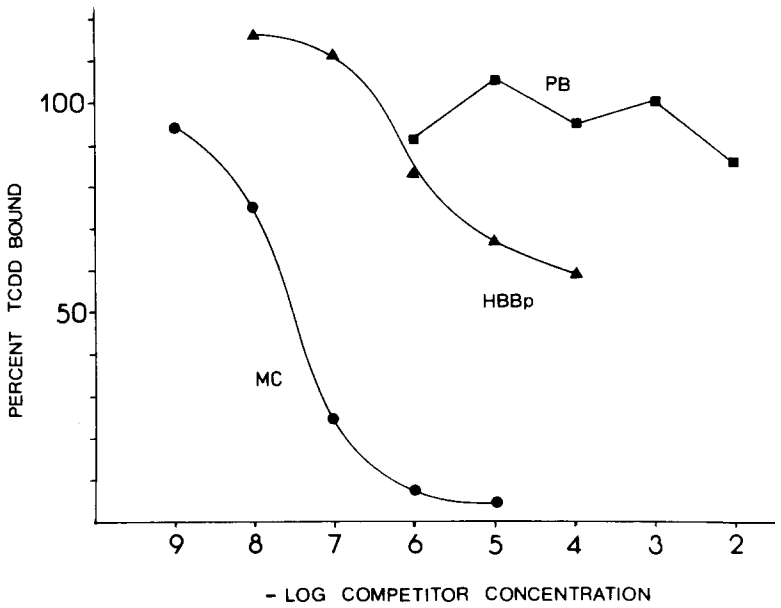

Fig. 4. Competition by PB, MC and HBBp for specific high affinity [^{3}H]TCDD binding sites. Hepatic cytosol was incubated at 5°C with 10 nm [^{3}H]TCDD for 1 h in the presence of varied concentrations of competitor. Specific binding of [^{3}H]TCDD at each competitor concentration was determined by the area under the specific binding peak produced by sucrose density gradient centrifugation after dextran-charcoal treatment. These results represent the determinations from one experiment. In other experiments, designed to test the reproducibility of the assay, it was found that the mean value of specific binding of [^{3}H]TCDD (as a percentage of control) in the presence of PB (10^{-6} M) was 102.2% (S.D. = 4.8).

DISCUSSION

For the PCBs and other classes of halogenated aromatic compounds, there is an excellent correlation between the toxicity of a congener and its ability to induce AHH [21,22]. In view of this correlation it is of interest to identify those PCB and PBB congeners which are potent inducers of this cytochrome *P*-448-dependent monooxygenase. The purpose of the present study was to examine the inductive effects of HBBp whose chloro analog, namely 2,3,3′,-4,4′,5-hexachlorobiphenyl, was shown in our laboratory to be potent inducer of AHH [8].

The ability of HBBp to (a) induce B[*a*]P and 4-CBP hydroxylase, (b) enhance the MC-inducible protein at Mr 55 000 on SDS polyacrylamide electrophoresis and (c) bind the Ah cytosolic receptor protein clearly indicate that this congener is a potent MC-type inducer of cytochrome *P*-448.

Since HBBp has been identified by G. Dannan and S.D. Aust (pers. comm.) as a minor component of fireMaster BP-6, the results of the present study suggest that HBBp is a likely contributor to the MC-type character of this

commercial PBB mixture. Although the present study did not permit a meaningful assessment of the toxicologic properties of HBBp, the chloro analog of HBBp, 2,3,3',4,4',5-hexachlorobiphenyl, causes thymic involution in the rat as well as the accumulation in the liver of triacylglycerols, cholesterol and phospholipids [23]. The toxic potency of 2,3,3',4,4',5-hexachlorobiphenyl has received particular attention since this congener (more than any other so far examined) is preferentially retained in Yusho patients [23]. The properties of the chloro analog of HBBp suggest that HBBp may contribute to the toxicologic properties as well as the MC-type character of fireMaster BP-6. For this reason the biologic and toxicologic characteristic of this and other PBB congeners are currently under investigation in our laboratory.

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