POLYCHLORINATED BIPHENYLS AS INDUCERS OF HEPATIC MICROSOMAL ENZYMES: EFFECTS OF DI-*ORTHO* SUBSTITUTION

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SUMMARY

All of the 13 possible polychlorinated biphenyl (PCB) isomers and congeners substituted at both *para* positions, at least two *meta* positions (but not necessarily on the same ring) and at two *ortho* positions have been synthesized and tested as rat hepatic microsomal enzyme inducers. The effects of these compounds were evaluated by measuring microsomal benzo[*a*]pyrene (B[*a*]P) hydroxylase, 4-chlorobiphenyl (4-CBP) hydroxylase, 4-dimethylaminoantipyrine (DMAP) *N*-demethylase and NADPH-cytochrome *c* reductase activities, the cytochrome b_5 content and the relative peak intensities and spectral shifts of the carbon monoxide(CO)- and ethyliso-cyanide(EIC)-difference spectra of ferrocytochrome *P*-450. The results were compared to the effects of administering phenobarbitone (PB), 3-methylcholanthrene (MC) and PB plus MC (coadministered). At dose levels of 150 μmol · kg^{-1}, all of the PCB congeners, except 2,3',4,4',5',6-hexachloro-biphenyl, significantly enhanced hepatic microsomal cytochrome *P*-450 content, B[*a*]P hydroxylase and/or DMAP *N*-demethylase activities compared to the control (corn oil-treated) animals. Only 5 of these compounds, namely 2,3,4,4',5,6-hexa-, 2,2',3,3',4,4'-hexa-, 2,2',3,4,4',5-hexa-, 2,3,3',4,4',6-hexa- and 2,2',3,3',4,4',5-heptachlorobiphenyl, enhanced microsomal B[*a*]P hydroxylase, 4-CBP hydroxylase, NADPH-cytochrome *c* reductase and DMAP *N*-demethylase activities in a manner consistent with a mixed pattern of induction. The results suggest that PCB isomers and congeners substituted at both *para* positions, at least two *meta* positions, at two *ortho* positions and containing a 2,3.4-trichloro substitution pattern on one ring are mixed-

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Abbreviations: B[*a*]P, benzo[*a*]pyrene; 4-CBP, 4-chlorobiphenyl; CO, carbon monoxide; DMAP, 4-dimethylaminoantipyrine; EC, electron capture; EIC, ethylisocyanide; GLC, gas liquid chromatography; MC, 3-methylcholanthrene; PB, phenobarbitone; PMR, proton magnetic resonance; TCDF, 2,3,7,8-tetrachlorodibenzofuran; TLC, thin-layer chromatography.

type inducers; in addition the effects of 2,3,4,4',5,6-hexachlorobiphenyl were also consistent with a mixed pattern of induction.

INTRODUCTION

Structure-activity rules for PCBs as inducers of hepatic microsomal enzyme activity in the immature male rat support the following correlations [1]: (1) There are no apparent rules for PB-type inducers due to the structurally-diverse PCB isomers and congeners which fall into this class of compounds. (2) MC-type inducers must be substituted at both *para* positions and at least two *meta* positions but not necessarily on the same phenyl ring. (3) With one exception, i.e., 2',3,4,4',5,5'-hexachlorobiphenyl, the substitution of one *ortho* substituent on the nucleus of the four possible MC-type inducers defined in (2) does not abolish this type of activity but give a series of mixed-type inducers.

The results obtained for 2',3,4,4',5,5'-hexachlorobiphenyl initially suggested that this compound was a PB-type inducer evidenced by enhanced hepatic microsomal B[a]P hydroxylase and DMAP N-demethylase activities and cytochrome P-450 content with negligible qualitative changes in the reduced cytochrome P-450/CO- and EIC-difference spectra compared to control rats [2]. However a more detailed study indicated that 2',3,4,4',5,5'-hexachlorobiphenyl is a novel inducer [3,4].

Previous reports have suggested that 3 PCB isomers, 2,2',4,4',5,5'-, 2,2',3,3',-4,4'- and 2,2',3',4,4',5-hexachlorobiphenyl, which contain the structural prerequisites defined in (2) plus two *ortho* substituents on different rings, may also be mixed-type inducers [5,6]. From a re-investigation of the effects of 2,2',4,4',5,5'-hexachlorobiphenyl, Goldstein et al. [7] concluded that this is a PB-type inducer and that the reported MC-type characteristics were due to trace contamination with highly active 2,3,7,8-tetrachlorodibenzofuran (TCDF) which was formed during the preparation of this compound via the copper-catalyzed Ullmann coupling of 2,4,5-trichloroiodobenzene. We have synthesized, purified and evaluated the effects of 2,2',3,3',4,4'- and 2,2',3',-4,4',5-hexachlorobiphenyl as microsomal enzyme inducers [8] and both compounds elicited responses consistent with mixed-type inducers as reported by Stonard and Greig [6].

This paper describes the synthesis and mode of microsomal enzyme induction of all those PCB isomers and congeners which possess the structural prerequisites for the strictly MC-type inducer (i.e., rule (2)) plus two *ortho* chloro substituents. The inductive effects of these 13 possible PCBs were compared to those elicited by PB, MC and PB + MC (coadministered) on microsomal B[a]P hydroxylase, 4-CBP hydroxylase, DMAP N-demethylase and NADPH cytochrome c reductase activities, the cytochrome b_5 content and the intensities and absorption maxima of the CO- and EIC-difference spectra of ferrocytochrome P-450. The structures of the strictly MC-type inducers (namely 3,4,4',5-tetra-, 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',-

PARENT
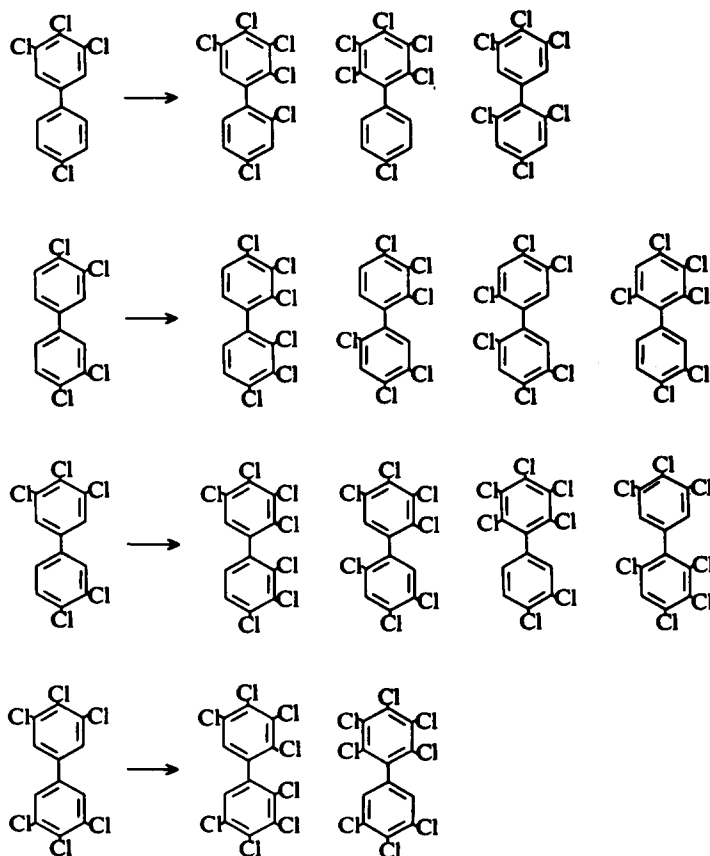MC-TYPE INDUCERS   DI-*ORTHO* DERIVATIVES

Fig. 1. Structures of the 13 possible di-*ortho* substituted derivatives derived from the 4 MC-type PCB inducers (the di-*ortho* compounds are shown in the same order as they appear in Tables I and II).

4,4',5,5'-hexachlorobiphenyl) and their 13 possible di-*ortho* derivatives are shown in Fig. 1.

MATERIALS AND METHODS

Synthesis and purification of PCB isomers and congeners

4-Chloroaniline, 2,4-dichloroaniline, 3,4-dichloroaniline, 2,3,4-trichloroaniline, 2,4,5-trichloroaniline, 3,4,5-trichloroaniline, 2,3,4,5-tetrachloroaniline, 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, 1,2,3,5-tetrachlorobenzene and pentachlorobenzene were purchased from the Aldrich Chemical Company and amyl nitrite was obtained from Pfaltz

TABLE I

SYNTHESIS AND PROPERTIES OF PCB ISOMERS AND CONGENERS

PCB congener	Synthetic precursors		GLC purity (%)	Molec. Ion	PMR chemical shifts
2,2',3,4,4',5-Hexachlorobiphenyl	2,4-dichloroaniline	1,2,3,4-tetrachlorobenzene	>98	358	7.33–7.70, M
2,3,4,4',5,6-Hexachlorobiphenyl	4-chloroaniline	pentachlorobenzene	>96	358	7.01 (H-2',6', d, J=8.3 Hz), 7.35 (H-3',5', d, J=8.8 Hz)
2,3',4,4',5',6-Hexachlorobiphenyl	3,4,5-trichloroaniline	1,3,5-trichlorobenzene	>98	358	7.59 (H-2',6', s), 7.66 (H-3,5, s)
2,2',3,3',4,4'-Hexachlorobiphenyl	2,3,4-trichloroiodobenzene		>99	358	7.39 (H-6,6', d, J=8.3 Hz), 7.74 (H-5,5', d, J=83. Hz)
2,2',3,4,4',5-Hexachlorobiphenyl	2,4,5-trichloroaniline	1,2,3-trichlorobenzene	>99	358	7.40 (H-6', d, J=8.5 Hz), 7.67 (H-6, s), 7.73 (H-5, d, J=8.5 Hz), 7.85 (H-3, s)
2,2',4,4',5,5'-Hexachlorobenzine	2,2',5,5'-tetrachlorobenzine		>99	358	7.70 (H-6,6', s), 7.91 (H-3,3', s)
2,3',4,4',5,6-Hexachlorobiphenyl	3,4-dichloroaniline	1,2,3,5-tetrachlorobenzene	>98	358	7.31 (H-6', q, J=8.4 Hz), 7.61 (H-2', d, J=2.0 Hz), 7.78 (H-5', d, J=8.4 Hz), 7.86 (H-5, s)
2,2',3,3',4,4',5-Heptachlorobiphenyl	2,3,4-trichloroaniline	1,2,3,4-tetrachlorobenzene	>96	392	7.44 (H-6, d, J=8.4 Hz), 7.71 (H-6, s), 7.79 (H-5', d, J=8.4 Hz)
2,2',3,4,4',5,5'-Heptachlrorobiphenyl	2,4,5-trichloroaniline	1,2,3,4-tetrachlorobenzene	>99	392	7.72 (H-6, s), 7.76 (H-6', s), 7.87 (H-3', s)
2,3,3',4,4',5,6-Heptachlorobiphenyl	3,4-dichloroaniline	pentachlorobenzene	>96	392	7.36 (H-6', q, J=1.9, 8.4 Hz), 7.63 (H-2', d, J=1.9 Hz), 7.79 (H-5, d, J=8.4 Hz)
2,3,3',4,4',5',6-Heptachlorobiphenyl	3,4,5-trichloroaniline	1,2,3,5-tetrachlorobenzene	>98	392	7.63 (H-2',6', s), 7.88 (H-5, s)
2,2',3,3',4,4',5,5'-Octachlorobiphenyl	2,3,4,5-tetrachlroroaniline	1,2,3,4-tetrachlorobenzene	>98	426	7.75 (H-6,6', s)
2,3,3',4,4',5,5',6-Octachlorobiphenyl	3,4,5-trichloroaniline	pentachlorobenzene	>96	426	7.66 (H-2',6', s)

and Bauer, Inc. 2,2',4,4',5,5'-Hexa- and 2,2',3,3',4,4'-hexachlorobiphenyl were synthesized from 2,2',5,5'-tetrachlorobenzidine and 2,3,4-trichloroiodo-benzene, respectively, as described [2,8]. The remaining PCB isomers and congeners were synthesized by the Cadogan coupling [9] of the appropriate chlorinated aniline (10—15 mmol) in excess chlorinated benzene (200—300 mmol) in the presence of a slight excess of amyl nitrite (20—25 mmol) as summarized in Table I. The reactions were performed at 110—125°C with rapid stirring for 18 h followed by removal of the excess chlorinated benzene in vacuo. The products were absorbed on neutral alumina which was added to a Florisil/neutral alumina column and the crude PCB product(s) eluted with petroleum spirit (b.p. 30—60°C). The crude product(s) were purified by preparative thin-layer chromatography (TLC) as described [10] and the purified congeners recrystallized from methanol and their structure confirmed by their mass and proton magnetic resonance (PMR) spectra as summarized in Table I. The gas-liquid chromatographic (GLC) purities were determined using a Hewlett-Packard model 5710 gas chromatograph equipped with a flame ionization or ^{63}Ni electron capture (EC) detector using an 0.6 cm X 1.2 m glass column packed with 3% OV 101 on Ultrabonded Carbowax 20M (80—100 mesh, RFR Corporation).

Biochemicals

Cytochrome *c* (horse heart, Type III) NADP$^+$, NADPH, glucose 6-phosphate, D-glucose-6-phosphate dehydrogenase (Baker's yeast), MC, B[a]P and EIC were purchased from Sigma Chemical Company; DMAP and CO were obtained from Aldrich Chemical and Matheson companies respectively and sodium PB was supplied by the dispensing pharmacy, Ontario Veterinary College, Guelph. [^{3}H]B[a]P was purchased from New England Nuclear, dissolved in hexane and washed with 0.5 M NaOH to extract oxidized products and further purified by Florisil column chromatography using petroleum ether (b.p. 30—60°C)/dichloromethane (99 : 1, v/v) as eluent. 4-[^{3}H]CBP was prepared by base-catalysed tritium exchange of 4-CBP (New England Nuclear). Like B[a]P, the 4-[^{3}H]CBP was washed with NaOH, passed through a Florisil column using hexane as eluent followed by TLC developed in chloroform/acetic acid (99 : 1, v/v).

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. The PCB isomers and congeners were dissolved in corn oil and doses of 150 μmol · kg^{-1} were administered (with one exception) to at least 4 animals by intraperitoneal injection on days 1 and 3. The animals were killed by cervical dislocation on day 6. 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 and 2 and the animals killed on day 3. Animals injected with a corresponding volume of 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 pre-weighed, 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 essentially as described [11].

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. [12]. The cytochrome P-450 content was determined by the method of Omura and Sato [13], 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 concentration 4.5 mM) instead of CO. The absence of a peak at 420 nm in the difference spectrum between carbonmonoxyhaemoglobin and oxyhaemoglobin (determined by bubbling CO through the sample cuvette using oxidised microsomes) indicated that the microsomes used in this study were essentially free from haemoglobin contamination. The concentration of cytochrome b_5 was determined from the difference spectrum between NADH-reduced microsomes and oxidised microsomes [14] using the corrected extinction coefficient of 185 cm^{-1} · mM^{-1} [13]. All spectra were recorded on a Cary 118C spectrophotometer with a repetitive scan accessory. Holmium oxide was used to calibrate all spectra.

All other assays were performed as described [11]. The rate of oxidative N-demethylation of DMAP was measured by quantifying the production of formaldehyde. The formaldehyde, trapped as the semicarbazone, was developed in double-strength Nash reagent. The rate of B[a]P hydroxylation [15,16] and 4-CBP hydroxylation [17] were both measured radiometrically by quantifying the base-soluble metabolites following hexane-extraction of the unreacted substrate. The activity of NADPH-cytochrome P-450 reductase was measured by the rate of reduction of cytochrome c. 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 values of mg microsomal protein g · $liver^{-1}$ given in Table II are expressed as an increase over control rather than the absolute value. This is because a new Potter-Elvehjem homogeniser was installed before completion of the experiments and this increased the yield, but not the specific activity, of the microsomal protein.

The statistical significance between sample means of control and treated

TABLE II

THE EFFECTS OF DI-ORTHO SUBSTITUTED PCB CONGENERS AS HEPATIC MICROSOMAL ENZYME INDUCERS IN MALE WISTAR RATS

*Significantly different from control, α = 0.01. Values are mean ± S.D.

Treatment	% Liver Wt. of Body Wt.	Mg Protein g liver[a]	DMAP N-demethylase[b]	NADPH-cytochrome c reductase[c]	Benzo[a]pyrene Hydroxylased	4-Chlorobiphenyl hydroxylased	Cytochrome b_5	Cytochrome P-450[e] (λmax, nm)	EIC-difference spectrum Peak maxima (nm)	Peak height (455/428) (nm)
Control (corn oil)	4.20±0.36	1.00±0.15	3.90±0.26	680±9.3	184±20	109±15	0.205±0.016	0.665±0.035 (450.0)	428.0, 455.0	0.51±0.03
PB	5.75±0.66*	1.41±0.05*	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	4.78±0.38*	1.17±0.08*	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	6.31±0.61*	1.47±0.07*	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*
2,2',3,4,4',5	5.78±0.71*	1.78±0.07*	9.78±0.41*	145±13*	287±19	127±6	0.288±0.010*	1.48±0.09* (450.0)	428.0, 455.0	0.65±0.02
2,3,4',5,6	6.96±0.80*	1.51±0.10*	11.0±0.73*	158±21*	967±106*	763±40*	0.293±0.021*	1.71±0.12* (449.8)	428.0, 454.6	0.71±0.09*
2,3',4,4',5,6	5.13±0.76	1.37±0.20*	4.13±0.63	74,3±8.2	184±18	130±55	0.230±0.024	0.605±0.056 (450.0)	428.0, 455.0	0.51±0.03
2,2',3,3',4,4'	4.28±0.04	1.25±0.13*	8.15±1.12*	138±14*	793±70*	689±85*	0.340±0.010*	1.08 ±0.12* (450.0)	428.3, 455.0	0.68±0.05*
2,2',3,4,4',5	5.34±0.50*	1.47±0.09*	9.58±0.82*	155±13*	1450±190*	913±87*	0.358±0.039*	1.68 ±0.08* (449.0)	428.7, 453.2	0.93±0.08*
2,2',4,4',5,5'	5.22±0.39*	1.67±0.11*	9.60±0.93*	165±13*	578± 63*	116±9	0.240±0.031*	1.66 ±0.15* (450.0)	428.0, 455.0	0.65±0.01
2,3,3',4,4',6	5.49±1.09*	1.29±0.06*	7.95±0.60*	135±15*	1070± 30*	837±40*	0.282±0.022*	1.37 ±0.11* (449.6)	428.6, 454.3	0.79±0.04*
2,2',3,3',4,4',5	5.04±0.30	1.29±0.03*	8.21±0.42*	143± 9*	1170±110*	757±140*	0.348±0.038*	1.65 ±0.07* (449.6)	428.3, 454.7	0.76±0.05*
2,2',3,4,4',5,5'	6.24±0.91*	1.28±0.05*	9.61±1.57*	150±11*	536±21*	98.7±6.2	0.325±0.010*	1.56 ±0.13* (450.0)	428.0, 455.0	0.58±0.01
2,3,3',4,4',5,6	6.63±0.42*	1.52±0.08*	9.14±0.42*	157±12*	547±71*	104± 18	0.310±0.002*	1.68 ±0.08* (450.0)	428.0, 455.0	0.57±0.01
2,3,3',4,4',5,6	5.70±0.12*	1.58±0.07*	7.68±0.67*	142±14*	666±84*	190± 30	0.293 ± 0.015	1.29 ±0.23* (449.7)	428.2, 454.5	0.71±0.06*
2,2',3,3',4,4',5,5'	5.52±0.63*	1.64±0.16*	8.00±0.95*	156±16*	153± 7	91.7±10.8	0.270±0.010*	1.23 ±0.13* (450.0)	428.0, 455.0	0.56±0.01
2,3,3',4,4',5,5',6	5.71±0.45*	1.36±0.12*	10.2±1.2*	163±15*	450± 21*	138± 13	0.288±0.019*	1.42 ±0.26* (450.2)	428.0, 455.0	0.49±0.04

[a] Increase over control.
[b] nmol HCHO formed mg · protein^{-1} · min^{-1}.
[c] nmol cytochrome c reduced · mg protein^{-1} · min^{-1}.
[d] pmol metabolized · mg protein^{-1} · min^{-1}.
[e] nmol · mg protein^{-1}.

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groups for each parameter studied was analysed by Dunnett's method for multiple comparisons with a control [18].

RESULTS

Synthesis of PCB congeners

The identification of TCDF as an impurity in the preparation of 2,2',-4,4',5,5'-hexachlorobiphenyl emphasises the need for synthetic routes which avoid the formation of active by-products. All of the crude reaction products used in this study were initially purified by chromatography through a Florisil/neutral alumina (approx. 1 : 1, w/w) column using petroleum ether (b.p. 30—60°C) as eluent. The Cadogan coupling reaction has been used in our studies as the major synthetic method and involves the generation of a reactive electrophile (and possibly a radical) via diazotization of a chlorinated aniline. Although dimerization of a 3,4,5-trichlorophenyl moiety would form the potent MC-type inducer 3,3',4,4',5,5'-hexachlorobiphenyl, the results suggest that this is not an important pathway since 2,3',4,4',5',6-hexachlorobiphenyl (synthetically derived from 3,4,5-trichloroaniline) does not show any significant activity as a microsomal enzyme inducer. Preparative TLC, the final chromatographic purification, is used to separate products and also ensures the removal of potential MC-type PCB and TCDF contaminants from the desired PCB product.

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

Induction of cytochromes P-450 by PB was characterised by (a) a proliferation of the endoplasmic reticulum (as indicated by an increase in the mg microsomal protein ⋅ g liver^{-1}), (b) an increase in the activity of DMAP N-demethylase ($\times 2.2$), B[a]P hydroxylase ($\times 3.1$) and NADPH-cytochrome c reductase ($\times 2.5$), (c) an increase in the concentration of cytochrome b_5 and P-450 with the absorption maximum of the latter occurring at 450.0 nm and (d) an EIC-difference spectrum displaying two peaks at 428.0 and 455.0 nm with a 455 : 428 nm peak height ratio of about 0.7. In contrast to all other parameters measured, PB treatment failed to significantly increase the activity of 4-CBP hydroxylase. These features are shown in Table II.

In contrast, induction of cytochromes P-448 by MC was characterised by (a) negligible proliferation of the endoplasmic reticulum and no significant increase in the activity of either DMAP-N-demethylase or NADPH-cytochrome c reductase, (b) large increases in the activities of B[a]P hydroxylase ($\times 15$) and 4-CBP hydroxylase ($\times 12$), (c) an increase in the concentration of cytochrome b_5 and P-450 with the absorption maximum of the latter shifted 2 nm downfield to 448.0 nm and (d) an EIC-difference spectrum with peak maxima at 429.6 and 450.0 nm and a '455 : 428' nm peak-height ratio of 1.9.

In all cases the coadministration of PB with MC resulted in increases in enzymic activity and cytochrome concentration which were slightly greater than the corresponding increases evoked by either PB or MC alone. Following

the coadministration of PB and MC, the CO-difference spectrum showed maximum absorbance at 448.5 nm, whilst the EIC-difference spectrum showed two peaks at 428.5 and 452.0 nm with a '455 : 428' nm peak height ratio of 1.2. The spectral shifts elicited by the coadministration of PB and MC were slightly less than those evoked by the administration of MC alone. In general, the qualitative spectral characteristics displayed by (PB + MC)-induced microsomes were intermediate between PB- and MC-induced microsomes.

The effects of PCB isomers and congeners

The results summarized in Table II indicate that at dose levels of 150 μmol · kg^{-1} only 2,3',4,4',5',6-hexachlorobiphenyl did not significantly increase the concentration of hepatic, microsomal cytochrome P-450. The 1.5- to 2.5-fold increase in cytochrome P-450 content induced by all of the remaining PCB congeners was accompanied by comparable increases in DMAP N-demethylase and NADPH-cytochrome c reductase activities. In other words, with the exception of the non-inducer 2,3',4,4',5',6-hexachlorobiphenyl, each PCB congener tested exhibited PB-type characteristics.

However, in contrast to PB, five of the PCB congeners, namely 2,3,4,4',-5,6-hexa-, 2,2',3,3',4,4'-hexa-, 2,2',3',4,4',5-hexa,- 2,3,3',4,4',6-hexa- and 2,2',3,3',4,4',5-heptachlorobiphenyl, induced 4-CBP hydroxylase by 6- to 8-fold over control. These five congeners similarly induced B[a]P hydroxylase more than PB. Furthermore these increases in 4-CBP and B[a]P hydroxylase activity were paralleled by shifts in the peak maxima of both the CO- and EIC-difference spectra. Therefore, in addition to their PB-type characteristics, these five PCB congeners also displayed varying degrees of MC-type characteristics.

DISCUSSION

Previous studies have shown that in the PCB series only 4 strictly MC-type inducers have been identified, namely, 3,3',4,4'-tetra-, 3,4,4',5-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl. With one exception, the substitution of one *ortho* chlorine on the nucleus of the MC-type inducers does not eliminate this type of activity and results in a series of mixed-type inducers. The exceptional PCB, 2',3,4,4',5,5'-hexachlorobiphenyl, has been categorised as a novel microsomal enzyme inducer [3,4] which contrasts with its bromo analogue, 2',3,4,4',5,5'-hexabromobiphenyl, which was classified as a mixed-type inducer [19]. There is no apparent explanation for this difference.

We have recently confirmed [8] that two PCB isomers, 2,2',3,4,4',5- and 2,2',3,3',4,4'-hexachlorobiphenyl, are also mixed-type inducers in contrast to the PB-type activity of 2,2',4,4',5,5'-hexachlorobiphenyl [7]. These three isomers are all di-*ortho* substituted PCBs derived from 3,3',4,4'-tetrachlorobiphenyl and this report compares the activity of all possible di-*ortho* sub-loss of the MC-type activity. A possible explanation of the results may be

stituted PCB congeners derived from the 4 MC-type inducers (see Fig. 1). The only di-*ortho* substituted PCB derived from 3,3′,4,4′-tetrachlorobiphenyl which had not been previously studied was 2,3,3′,4,4′,6-hexachlorobiphenyl in which both *ortho* chloro substituents are on the same phenyl ring. This isomer enhanced microsomal NADPH-cytochrome *c* reductase, 4-CBP hydroxylase, B[*a*]P hydroxylase and DMAP *N*-demethylase activities consistent with a mixed-type induction pattern. The results indicate that for this series of PCB isomers, only those isomers which contain an isolated 2,3,4-trichlorophenyl substitution are mixed-type inducers and apparently the second *ortho* substituent can appear on either phenyl ring.

There are three possible di-*ortho* substituted isomers of 3,4,4′,5-tetrachlorobiphenyl, namely 2,3′,4,4′,5′,6-, 2,2′,3,4,4′,5- and 2,3,4,4′,5,6-hexachlorobiphenyl, and only the latter isomer elicited effects consistent with a mixed-type inducer. The effects of structure in this series are remarkably illustrated by the successive transfer of the *ortho* chloro groups in 2,3,4,4′,-5,6-hexachlorobiphenyl to the less substituted ring. Transfer of one *ortho* chloro group from this mixed inducer gives the 2,2′,3,4,4′,5-isomer, which is a PB-type inducer, and transfer of the second *ortho* group gives the 2,3′,4,4′,5′,6-isomer, which is the only PCB in the series which had no significant effect on any of the enzymic or spectral parameters reported in Table II.

The four di-*ortho* substituted heptachlorobiphenyls and two di-*ortho* substituted octachlorobiphenyls derived from 3,3′,4,4′,5-penta- and 3,3′,4,4′,5,5′,-hexachlorobiphenyl, respectively, all enhanced hepatic microsomal DMAP-*N*-demethylase ($\gtrsim$2-fold), NADPH-cytochrome *c* reductase (>2-fold) and B[*a*]P hydroxylase activity (>2-fold). With the exception of the asymmetrical 2,2′,3,3′,4,4′,5-heptachlorobiphenyl, these congeners could not be distinguished from PB in their mode of induction. 2,2′,3,3′,4,4′,5-Heptachlorobiphenyl was the only inducer in this series to enhance both B[*a*]P hydroxylase (~2X PB) and 4-CBP hydroxylase (6X PB) activities consistent with a mixed-type induction pattern.

The results indicate that the introduction of two *ortho* substituents to the nucleus of the four MC-type inducers does not eliminate this activity if one ring has a 2,3,4-trichloro substitution pattern. The only exception to this rule is 2,3,4,4′,5,6-hexachlorobiphenyl which also elicited responses typical of this class of inducers. These relationships emphasize both the unusual properties of the 2,3,4-trichlorophenyl moiety and the myriad of subtle effects of structure on the activity of PCB isomers and congeners as hepatic microsomal enzyme inducers. One such example illustrates the effects of *meta* substituents: 2,2′,3,3′,4,4′,5-heptachlorobiphenyl is a mixed-type inducer but can be converted to a PB-type inducer either by eliminating the 3′-substituent to give 2,2′,3,4,4′,5-hexachlorobiphenyl or by its transfer to the 5′-*meta* position to give 2,2′,3,4,4′,5,5′-heptachlorobiphenyl. Similarly, although 2,3,4,4′,5,6-hexachlorobiphenyl is a mixed-type inducer, the addition of one or two *meta* chloro substituents to give 2,3,3′,4,4′,5,6-hepta- and 2,3,3′,4,4′,5,5′,6-octachlorobiphenyl, respectively, results in the

loss of the MC-type activity. A possible explanation of the results may be associated with the stereochemical conformations of these compounds in solution which would presumably affect their capacities to bind to the cytosolic receptor protein which plays a critical role in hepatic microsomal enzyme induction [20—22]. The crystal structures, conformational properties and the receptor-binding characteristics of these PCB congeners are currently being investigated.

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