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Chlorinated Biphenyl Induction of Aryl Hydrocarbon Hydroxylase Activity: a Study of the Structure-Activity Relationship

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SUMMARY

POLAND, ALAN & GLOVER, EDWARD (1977) Chlorinated biphenyl induction of aryl hydrocarbon hydroxylase activity: a study of the structure-activity relationship. *Mol. Pharmacol.*, 13, 924-938.

Of 16 halogenated biphenyls examined for their capacity to induce hepatic aryl hydrocarbon hydroxylase activity in the chicken embryo, only three congeners, 3,4,3′,4′-tetrachlorobiphenyl, 3,4,5,3′,4′,5′-hexachlorobiphenyl, and 3,4,5,3′,4′,5′-hexabromobiphenyl, were active. They were approximately equipotent in the chicken embryo, with ED_{50} values of approximately 90 nmoles/kg. These three chlorobiphenyl congeners also induced hepatic aryl hydrocarbon hydroxylase activity in C57BL/6J mice and rats, and they competitively bound the hepatic cytosol binding species thought to be the receptor for the induction of this enzyme. Among the halogenated biphenyls, there appear to be two structural requirements for induction of aryl hydrocarbon hydroxylase activity: the presence of at least 2 adjacent halogen atoms in the lateral positions of each benzene ring (positions 3,4,3′,4′ or 3,4,5,3′,4′,5′) and the absence of halogen atoms adjacent to the biphenyl bridge (positions 2,6,2′, and 6′); such substitutions lead to marked nonplanarity of the molecule. The halobiphenyl congeners that induced aryl hydrocarbon hydroxylase activity did not induce aminopyrine *N*-demethylase activity (a measure of phenobarbital-like activity); however, some of the congeners that failed to induce aryl hydrocarbon hydroxylase activity did induce aminopyrine *N*-demethylase. This suggests that while the commercial mixture of chlorobiphenyls, Aroclor 1254, induces a mixed pattern of microsomal monooxygenase activity resembling the combined administration of 3-methylcholanthrene and phenobarbital, any given chlorobiphenyl congener appears to produce only a 3-methylcholanthrene-like or a phenobarbital-like response (or the congener may be devoid of both activities). 3,4,3′,4′-Tetrachlorobiphenyl, the prototype of the halobiphenyls that induce hepatic aryl hydrocarbon hydroxylase activity, is an approximate isostereomer of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran, and 3,4,3′,4′-tetrachloroazoxybenzene, all of which induce hepatic aryl hydrocarbon hydroxylase activity but which are over 100-fold more potent. Conversion of 3,4,3′,4′-tetrachlorobiphenyl to a more rigid, planar analogue, 2,3,6,7-tetrachlorobiphenylene, produced a compound roughly equipotent to TCDD in inducing hepatic aryl hydrocarbon hydroxylase activity in the chicken embryo.

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INTRODUCTION

The hepatic microsomal monooxygenase system (also known as hepatic microsomal drug-metabolizing enzymes) metabolizes many drugs and other foreign compounds. This enzyme complex consists of NADPH–cytochrome P-450 reductase and cytochrome P-450, the terminal component, which contains the enzyme active site and determines substrate specificity. There are several distinct subspecies of cytochrome P-450 in liver microsomes, which have different substrate specificity and are differentially induced[2] by the administration of a number of compounds (2). Compounds that induce hepatic microsomal monooxygenase activities can be divided into two major classes: one typified by phenobarbital, which induces several subspecies of cytochrome P-450 and associated monooxygenase activities directed toward a wide variety of substrates; and the other, by 3-methylcholanthrene, which induces a distinct subspecies of cytochrome P-450 and associated enzyme activities for a more limited group of substrates (3, 4).

A distinct pattern of induction of hepatic microsomal monooxygenase activity is produced by polychlorinated biphenyls (5), industrial chemicals that are widely used as heat-exchange fluids, lubricants, insulators, and plasticizers (6). The persistence of these compounds in the environment, and their accumulation in human and animal tissues, has raised concern about their potential health hazard (6). Alvares *et al.* (5) noted that the administration of a commercial mixture of chlorinated biphenyl isomers, Aroclor 1254,[3] produced a complex pattern of induction of microsomal monooxygenase activity in rat liver, comparable to that produced by the combined administration of phenobarbital and MC.[4] Aroclor 1254 induced ethylmorphine *N*-demethylase, a phenobarbital-inducible monooxygenase activity, and aryl hydrocarbon hydroxylase, an MC-inducible activity. The administration of a commercial mixture of brominated biphenyls, Firemaster BP-6 (a fire retardant), produced a similar complex pattern of enzyme induction (12). Recently Ryan *et al.* (13, 14) reported the purification of the major cytochrome P-450 subspecies induced by Aroclor 1254 in rat liver. One was electrophoretically and antigenically very similar to, if not identical with, the major type of cytochrome P-450 purified from phenobarbital-treated rats, and the other corresponded to cytochrome P_1-450, the major subspecies purified from MC-treated rats.

We have considered the structure-activity relationships among chlorobiphenyl congeners that induce microsomal monooxygenase activity, with particular interest in the congeners that induce aryl hydrocarbon hydroxylase activity. There have been several previous reports of the effects of chlorinated biphenyl isomers on hepatic microsomal monooxygenase activity (15–18), but no clear-cut structure-activity relationship has emerged. A question of particular interest is: Does any given chlorinated biphenyl isomer have the capacity to induce hepatic microsomal cytochromes P-450 and associated monooxygenase activities in the complex pattern of Aroclor 1254, or will any given isomer induce only phenobarbital-like or MC-like patterns? This question is of considerable theoretical interest, as outlined below.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin is a potent inducer of hepatic cytochrome P_1-

[2] Induction of a microsomal monooxygenase activity or cytochrome P-450 subspecies denotes a relative increase in the enzyme activity or difference spectrum of the cytochrome, without implying a mechanism. In the case of induction of aryl hydrocarbon hydroxylase activity and cytochrome P_1-450, the evidence suggests that this increase is due to protein synthesis of cytochrome P_1-450 *de novo* and does not result from either a decrease in the degradation of the hemoprotein or activation of a preexisting membrane component (1).

[3] Aroclor 1254 is a mixture of chlorinated biphenyl isomers, manufactured by Monsanto, which is 54% chlorine by weight (on average pentachlorinated biphenyl) (7). Commercial mixtures of chlorinated biphenyls, such as Aroclor 1254, have been shown to contain trace amounts of chlorinated naphthalenes and dibenzofurans (8–10), and certain of the latter are potent inducers of cytochrome P_1-450 and aryl hydrocarbon hydroxylase activity (11).

[4] The abbreviations used are: MC, 3-methylcholanthrene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

450 and aryl hydrocarbon hydroxylase activity, resembling MC but 30,000 times as potent (19). Using tritiated TCDD, we have recently identified a binding species in hepatic cytosol of rats and mice which has the binding properties *in vitro* predicted for the recognition site or receptor for induction of cytochrome P_1-450 or aryl hydrocarbon hydroxylase activity (11). This protein in hepatic cytosol reversibly binds [^{3}H]TCDD with high affinity, and the binding affinities of other chlorinated dibenzo-*p*-dioxin congeners correspond rather closely to their biological potencies in inducing hepatic aryl hydrocarbon hydroxylase activity. Polycyclic aromatic hydrocarbons which induce cytochrome P_1-450 and the hydroxylase activity bind to this cytosol protein, while compounds that do not induce or, like phenobarbital, induce a different pattern of microsomal monooxygenase activity show no affinity for this cytosol protein. Thus the evidence suggests that this binding species in hepatic cytosol is the receptor site for the expression of cytochrome P_1-450 and aryl hydrocarbon hydroxylase activity. By analogy, one might hypothesize that there is a similar recognition site for phenobarbital and phenobarbital-like drugs which controls the induction of cytochrome P-450 and its associated monooxygenase activities.

This study was undertaken to determine whether any chlorinated biphenyl congener will bind to both putative receptor sites, or whether a given chlorinated biphenyl congener will bind only to the TCDD (or MC) receptor or only to the hypothetical receptor for phenobarbital. We approached this problem by asking which of a series of chlorinated biphenyl isomers will induce hepatic aryl hydrocarbon hydroxylase activity in the chicken embryo and bind to the cytosol binding species in mouse liver. We then examined whether any of these chlorobiphenyl congeners with MC-like activity could also evoke a phenobarbital-like response in mouse or rat liver.

MATERIALS AND METHODS

Materials

Biphenyl was purchased from Eastman Organic Chemicals; 1-chlorobiphenyl, 3,4-dichlorobiphenyl, 4,4'-dichlorobiphenyl, 2,6,2',6'-tetrachlorobiphenyl, 2,4,3',4'-tetrachlorobiphenyl, 2,3,2',3'-tetrachlorobiphenyl, and 2,3,4,2',3',4'-hexachlorobiphenyl, from Analabs, Inc.; 3,5,3',5'-tetrabromobiphenyl, 2,4,5,2',4',5'-hexachlorobiphenyl, 2,4,6,2',4',6'-hexachlorobiphenyl, 3,4,5,3',4',5'-hexabromobiphenyl, decachlorobiphenyl, 4,4'-dichloro-3,3'-dihydroxybiphenyl, and 3,5,3',5'-tetrachloro-4,4'-dihydroxybiphenyl from En Chem Environmental Division, RFR Corporation, Hope, R. I.; and 3,4,3',4'-tetrachlorobiphenyl, from both Analabs and RFR. Dr. James McKinney, National Institute of Environmental Health Sciences, and Dr. Joyce Goldstein, Environmental Protection Agency, Research Triangle Park, N. C., generously provided samples of 3,5,3',5'-tetrachlorobiphenyl and 3,4,5,-3',4',5'-hexachlorobiphenyl. Dr. John F. W. McOmie, Department of Chemistry, University of Bristol, England, generously provided 3,4,3',4'-tetramethoxybiphenyl, 3,4,5,3',4',5'-hexamethoxybiphenyl, 2,3,6,7-tetrachlorobiphenylene, 2,3,6,7-tetrabromobiphenylene, 2,3,6,7-tetramethoxybiphenylene, and 2,7-dinitrobiphenylene (20). Dr. R. F. C. Brown, Department of Chemistry, Australian National University, Canberra, generously provided octachlorobiphenylene (20). Aroclor 1254 (lot AK-38) was a sample from Monsanto.

The compounds that were biologically active in inducing hepatic aryl hydrocarbon hydroxylase activity (3,4,3',4'-tetrachlorobiphenyl, 3,4,5,3',4',5'-hexachlorobiphenyl, 3,4,5,3',4',5'-hexabromobiphenyl, 2,3,6,7-tetrachlorobiphenylene, and 2,3,6,7-tetrabromobiphenylene) were analyzed for purity by mass spectroscopy by Dr. George Kuta, Department of Chemistry, University of Rochester. The fragmentation patterns of each of these compounds suggested 99% or greater purity, but would not have detected isomeric impurities of the same molecular weight.

NADPH, NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase (*Torula* XI), semicarbazide, Tris, and dithiothreitol were purchased from Sigma Chemical Company. Sodium phenobarbital was obtained from Merck, and 3-meth-

ylcholanthrene, from K&K.

Radiolabeled TCDD. [1,6-^{3}H]2,3,7,8-Tetrachlorodibenzo-*p*-dioxin was prepared as described previously (11). Two different batches were synthesized: (a) 52.5 Ci/mmole, 93% pure by gas chromatography with 7% [1,6-^{3}H]2,3,7-trichlorodibenzo-*p*-dioxin, and (b) 36.7 Ci/mmole, 83% pure by gas chromatography and containing 17% of the tritiated trichloro analogue. Since the binding affinity of 2,3,7-trichlorodibenzo-*p*-dioxin is only one-seventh of that of TCDD (11), the presence of this impurity is of little significance.

Animals. Fertilized white Leghorn chicken eggs were purchased from Truslow Farms, Chestertown, Md., and incubated at 37° with at least 70% humidity. Male Sprague-Dawley rats (50–70 g) were purchased from Blue Spruce Farms, Altamont, N. Y., and C57BL/6J and DBA/2J mice were purchased from the Jackson Laboratory, Bar Harbor, Me. The animals were housed and fed as described previously (11).

All biphenyl and biphenylene compounds and TCDD were dissolved in *p*-dioxane, and 25 μl of solution were administred into the air sac of the egg through a small hole punched in the shell. 3-Methylcholanthrene was dissolved in dimethyl sulfoxide, and 50 μl were injected into the yolk sac. Rats and mice were injected intraperitoneally. The biphenyl congeners were dissolved in corn oil (8 ml/kg), TCDD was dissolved in *p*-dioxane (0.4 ml/kg), and sodium phenobarbital was dissolved in 0.9% NaCl (10 ml/kg). The dosage regimens are described in the legends to the tables and figures.

Tissue preparation. The chicken embryos, mice, or rats were killed, and their livers were weighed, homogenized in cold isotonic potassium chloride or 0.2 M potassium phosphate, and centrifuged at 10,000 $\times$ *g* for 20 min. The 10,000 $\times$ *g* supernatant fraction was used for enzyme assays. To prepare microsomes, the postmitochondrial supernatant fraction was centrifuged at 105,000 $\times$ *g* for 1 hr, resuspended, and resedimented.

Enzyme Assays

Aryl hydrocarbon hydroxylase activity. The 10,000 $\times$ *g* supernatant fraction of liver was assayed by the method of Gielen *et al.* (21), modified slightly as previously described (19). Enzyme activity was expressed as picomoles of 3-hydroxybenzo[*a*]pyrene formed per milligram of liver, wet weight, per minute of incubation.

Aminopyrine N-demethylase. The activity of this enzyme was measured in rat liver by the radiometric assay previously described (22) and modified for rat liver (8). The 10,000 $\times$ *g* supernatant fraction of rat liver, equivalent to 5 or 10 mg of liver, wet weight, was incubated with cofactors and 2 mM aminopyrine containing 5×10^5 dpm of [^{14}C]dimethylaminopyrine for 10 min. Enzyme activity was expressed as picomoles of [^{14}C]formaldehyde formed per milligram of liver, wet weight, per minute.

Cytochrome P-450. The washed microsomal pellet of chicken embryo, mouse liver, or rat liver was resuspended in 0.1 M potassium phosphate buffer (pH 7.4) containing 20% (v/v) glycerol. Cytochrome P-450 was measured by the method of Omura and Sato (23), as previously described (8).

Protein. Protein concentrations were determined by the method of Lowry *et al.* (24), using bovine serum albumin as standard.

Competitive Binding of Biphenyl and Biphenylene Congeners for Specific Binding Sites of [^{3}H]TCDD in Hepatic Cytosol

The affinity of halogenated biphenyls and biphenylenes for the specific binding sites in hepatic cytosol of C57BL/6J mice was determined by their capacity to compete with [^{3}H]TCDD for these sites, as previously described (11). The data were then plotted as the logarithm of the concentration of the competing congener vs. the percentage of the control specific binding of [^{3}H]TCDD (Fig. 2), and the dose of the biphenyl or biphenylene congener which reduced the specific binding of [^{3}H]TCDD by one-half was estimated. The relative binding affinity of these compounds is related by the equation $K_A/K_B = [A]/[B]$, where K_A and K_B are the equilibrium dissociation constants of compounds *A* and *B*, and [*A*] and [*B*] are the concentrations

of the free compounds which reduce [^{3}H]TCDD binding by 50%. In estimating the binding affinity of compounds using this equation, we make the assumption that [free ligand] = [total ligand]. With compounds having a high affinity ($K_D \leqq 1$ nM), this assumption is not fully justified and introduces a modest error. (For a fuller discussion, see ref. 11, footnote 4.)

Liquid Scintillation Counting

Aqueous samples containing [^{3}H]TCDD (0.4 or 0.6 ml) or [^{14}C]formaldehyde (0.5 ml) were added to 5 ml of scintillation mixture, and the radioactivity was quantified in a Beckman LS-330 scintillation counter. The scintillation mixture consisted of 1 liter of toluene, 500 ml of Triton X-100, 8.25 g of 2,5-diphenyloxazole, and 0.18 g of 1,4-bis[2-(5-phenyloxyazolyl)]benzene. Quenching was corrected by automatic external standardization. The efficiency of counting tritium was 36%, and the efficiency of counting ^{14}C was 93%.

RESULTS

Sixteen chlorinated and brominated biphenyl compounds were tested for their capacity to induce hepatic aryl hydrocarbon hydroxylase activity in the chicken embryo. At each dose tested, a compound was administered to the chicken embryos, and enzyme activity was measured 24 hr later in four groups of four pooled livers. Every experiment included a control group of eggs injected only with solvent (0% response) and a maximally induced group injected with 0.155 nmole of TCDD per egg (100% response). The maximal response produced by TCDD was approximately a 10-fold increase in enzyme activity. Halogenated biphenyls were considered inactive if at a dose of 466 nmoles/egg they evoked less than 10% of the maximal response. Active compounds were tested at four different dose levels, and from the log dose-response curve, the dose that induced hepatic aryl hydrocarbon hydroxylase activity to one-half the maximal response (ED_{50}) was estimated.

As shown in Table 1, only three of the 16 compounds induced enzyme activity—3,4,3′,4′-tetrachlorobiphenyl (*9*), 3,4,5,-3′,4′,5′-hexachlorobiphenyl (*10*), and 3,4,5,3′,4′,5′-hexabromobiphenyl (*11*)—and they were approximately equipotent, with ED_{50} values of 4.4, 4.7, and 3.4 nmoles/egg, respectively.[5] Comparing these three compounds with the rest of the halogenated biphenyls in Table 1, one can note two structural requirements which appear to be necessary for induction of aryl hydrocarbon hydroxylase activity. (a) The active compounds have adjacent halogen atoms in at least two of the lateral positions of each benzene ring (3,4,3′,4′ or 3,4,5,3′,4′,5′). Note that the 3,5,3′,5′-tetrahalobiphenyls (compounds *8* and *16*) were inactive. (b) The biologically active congeners have no halogen substitutions in positions adjacent to the biphenyl bridge (the 2, 6, 2′, or 6′ position); such substitutions produce marked nonplanarity of the two rings. Note that 2,4,3′,4′-tetrachlorobiphenyl (*6*), 2,3,4,2′,3′,4′-hexachlorobiphenyl (*12*), and 2,4,5,2′,4′,5′-hexachlorobiphenyl (*13*) were all inactive.

Substitution of other electron-withdrawing groups in the lateral ring positions resulted in inactive analogues. The following compounds did not induce hepatic aryl hydrocarbon hydroxylase activity at 466 nmoles/egg: 3,4,3′,4′-tetramethoxybiphenyl (*17*), 3,4,5,3′,4′,5′-hexamethoxybiphenyl (*18*), 4,4′-dichloro-3,3′-dihydroxybiphenyl (*19*), and 3,5,3′,5′-tetrachloro-4,4′-dihydroxybiphenyl (*20*).

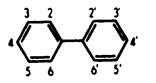
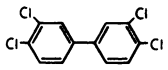
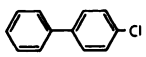
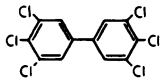
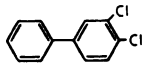
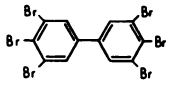
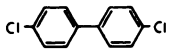
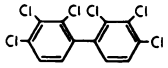
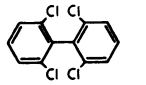
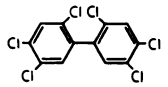
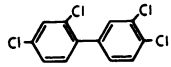
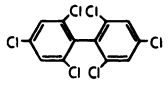
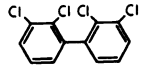
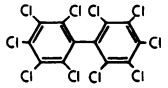
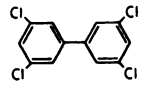
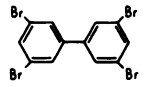
Aroclor 1254, the commercial mixture of chlorobiphenyl isomers, was quite a weak inducer of hepatic aryl hydrocarbon hydroxylase activity in the chicken embryo, with an ED_{50} of at least 20 μmoles/egg. We adopted 3,4,3′,4′-tetrachlorobiphenyl (*9*) as the prototype of the congeners that induce hepatic aryl hydrocarbon hydroxylase activity because the hexachlorobiphenyl analogue is not commercially available. It is useful to compare the potency of 3,4,3′,4′-tetrachlorobiphenyl (ED_{50} = 4.4 nmoles/egg) with that of TCDD (ED_{50} = 10 pmoles/egg) (25) and with that of Aroclor 1254 ($ED_{50} \geqq 20$ μmoles/egg). TCDD is almost 500 times as

[5] To convert nanomoles per egg to nanomoles per kilogram, we assume that the average chicken egg weighs 50 g, and multiply nanomoles per egg by 20.

TABLE 1

Potency of halogenated biphenyls in inducing hepatic aryl hydrocarbon hydroxylase activity in chicken embryos

Chicken embryos at 17 days of gestation were administered halogenated biphenyl compounds dissolved in 25 μl of *p*-dioxane. Twenty-four hours later they were killed and their livers were assayed for aryl hydrocarbon hydroxylase activity. The biologically active congeners were tested at four doses, and the ED_{50} was estimated from the log dose-response curve generated.

		ED_{50} (mol/egg)			ED_{50} (mol/egg)
1		INACTIVE	9		4.4×10^{-9}
2		INACTIVE	10		4.7×10^{-9}
3		INACTIVE	11		3.4×10^{-9}
4		INACTIVE	12		INACTIVE
5		INACTIVE	13		INACTIVE
6		INACTIVE	14		INACTIVE
7		INACTIVE	15		INACTIVE
8		INACTIVE	16		INACTIVE

potent as 3,4,3′,4′-tetrachlorobiphenyl and 2×10^5 times as potent as the commercial chlorobiphenyl mixture.

A comparison of the effects of maximal or nearly maximal inducing doses of 3,4,3′,4′-tetrachlorobiphenyl and TCDD on the induction of hepatic aryl hydrocarbon hydroxylase activity and cytochrome P_1-450 is shown in Table 2. The two compounds produced a comparable induction of enzyme activity, both produced a nearly 3-fold increase in total cytochrome P-450, and both produced a 2 nm shift in the CO absorption maximum of the cytochrome. It is of note that in the chicken embryo the absorption maxima for both control cytochrome P-450 and cytochrome P_1-450 were 2 nm greater than the respective values reported in rodents (26).

As shown in Fig. 1, 3,4,3′,4′-tetrachlorobiphenyl and TCDD are approximate isostereomers; 3,4,3′,4′-tetrachlorobiphenyl can assume a planar or nearly planar configuration, with its 4 chlorine atoms projecting from the lateral ring positions quite similarly to TCDD. However, if chlorine is substituted in a position adjacent to the biphenyl bridge (e.g., 2,4,3′,4′-tetrachlorobiphenyl, shown in Fig. 1), this sterically hinders the biphenyl rings and prevents planarity. As shown in Table 1, such nonplanar chlorobiphenyl analogues are inactive in inducing aryl hydrocarbon hydroxylase activity.

We previously reported that TCDD, 2,3,7,8-tetrachlorodibenzofuran, and 3,4,3′,4′-tetrachloroazoxybenzene are approximate stereoisomers and are approxi-

TABLE 2

Induction of hepatic aryl hydrocarbon hydroxylase activity and cytochrome P-450

Chicken embryos at 17 days of gestation were injected with 25 μl of *p*-dioxane or the same volume of solvent containing TCDD (0.155 nmole/egg) or 3,4,3',4'-tetrachlorobiphenyl (46.6 nmoles/egg). Forty-eight hours later the embryos were killed, and the livers of four like-treated animals were pooled and assayed for aryl hydrocarbon hydroxylase activity and cytochrome P-450. Each value is the mean ± standard error for four groups of four pooled livers.

Treatment	Aryl hydrocarbon hydroxylase activity	Cytochrome P-450	Absorption maximum[a]
	pmoles/mg liver/min	*nmole/mg protein*	*nm*
Control	0.60 ± 0.04	0.232 ± 0.026	451.7 ± 0.2
TCDD	22.7 ± 0.5	0.680 ± 0.053	449.7 ± 0.1
3,4,3',4'-Tetrachlorobiphenyl	17.0 ± 1.7	0.647 ± 0.058	449.6 ± 0.2

[a] Absorption maximum of the CO-reduced vs. reduced difference spectrum of microsomes.

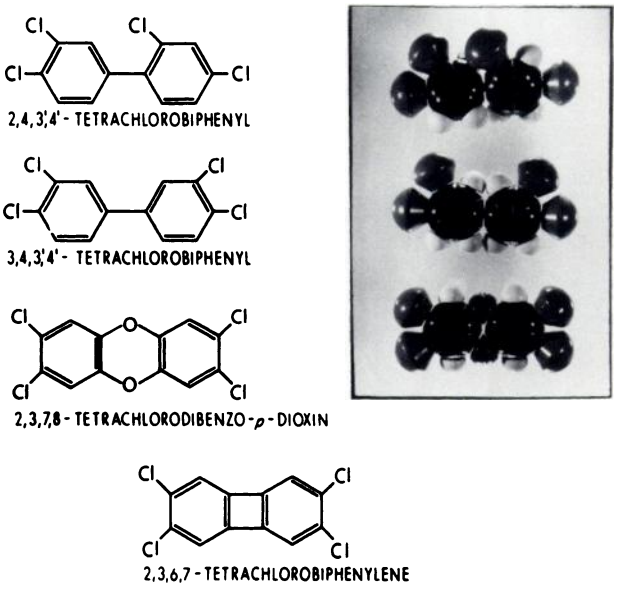


FIG. 1. *Comparison of chemical structures and molecular models of 2,4,3',4'-tetrachlorobiphenyl, 3,4,3',4'-tetrachlorobiphenyl, TCDD, and 2,3,6,7-tetrachlorobiphenylene*

No molecular model is shown for 2,3,6,7-tetrachlorobiphenylene because it was impossible to construct the strained ring system.

mately equipotent in inducing hepatic aryl hydrocarbon hydroxylase activity in the chicken embryo (27). 3,4,3',4'-Tetrachlorobiphenyl is roughly isosteric with these compounds, but 200 times less potent. If one could fix the biphenyl rings into a more rigid and planar configuration, such as 2,3,6,7-tetrachlorobiphenylene (Fig. 1), one might anticipate a considerably more potent compound.

2,3,6,7-Tetrachlorobiphenylene was found to be a very potent inducer of hepatic aryl hydrocarbon hydroxylase activity (ED_{50} = 14 pmoles/egg), as was 2,3,6,7-tetrabromobiphenylene (ED_{50} = 22 pmoles/egg). Both these biphenylenes were approximately equipotent to TCDD, 2,3,7,8-tetrachlorodibenzofuran, and 3,4,-3',4'-tetrachloroazoxybenzene. Octachlorobiphenylene, 2,3,6,7-tetramethoxybi-

phenylene, and 2,7-dinitrobiphenylene were inactive in inducing hepatic aryl hydrocarbon hydroxylase activity at doses of 46 nmoles/egg. While this structure-activity relationship for the biphenylenes is limited, it is remarkably similar to that of the halogenated dibenzo-*p*-dioxins and dibenzofurans (11, 25).

Binding to Hepatic Cytosol in Vitro

We have recently identified a binding species in the hepatic cytosol of C57BL/6J mice which has the binding properties *in vitro* predicted for the receptor for induction of aryl hydrocarbon hydroxylase activity (11). This hepatic cytosol protein reversibly binds [^{3}H]TCDD with high affinity, and the binding affinities of TCDD, 2,3,7,8-tetrachlorodibenzofuran, 3,4,3',4'-tetrachloroazoxybenzene, and their congeners correlate well with the potencies of these compounds in inducing hepatic enzyme activity in the chicken embryo (11, 27). If 3,4,3',4'-tetrachlorobiphenyl and 2,3,6,7-tetrachlorobiphenylene induce aryl hydrocarbon hydroxylase activity by the same mechanism as TCDD, they should bind to the same cytosol binding species.

The capacity of biphenyl and biphenylene congeners to compete for the stereospecific binding of [^{3}H]TCDD to mouse liver cytosol is shown in Fig. 2. Both 2,3,6,7-tetrachlorobiphenylene and 2,3,6,7-tetrabromobiphenylene bound avidly, while 3,4,3',4'-tetrachlorobiphenylene was about 30 times less potent. Octachlorobiphenylene and 2,4,5,2',4',5'-hexachlorobiphenyl, which were biologically inactive in inducing aryl hydrocarbon hydroxylase activity, failed to compete with [^{3}H]TCDD for specific binding sites, even when present at 1000 times the concentration of the radioligand.

From the concentration of the unlabeled congener necessary to compete for one-half the specific binding of [^{3}H]TCDD and the concentration of [^{3}H]TCDD, one can calculate the binding affinity (K_D = equilibrium dissociation constant) for each of these congeners for the hepatic cytosol binding species. Table 3 compares the binding affinity *in vitro*, K_D (for mouse liver cytosol), and the biological potency *in vivo*, ED_{50} (for induction of hepatic aryl hydrocarbon hydroxylase activity in the chicken embryo), for several biphenylene and one biphenyl congener. Compounds were considered biologically inactive if the highest dose tested produced less than 10% of maximal induction, and congeners were considered inactive *in vitro* if the highest concentration judged to be in solution produced less than a 20% decrease in the specific binding of [^{3}H]TCDD. For inactive compounds the highest dose tested (nanomoles per kilogram) or highest concentration (nanomolar) is given in parentheses.

It can be seen that 2,3,6,7-tetrachlorobi-

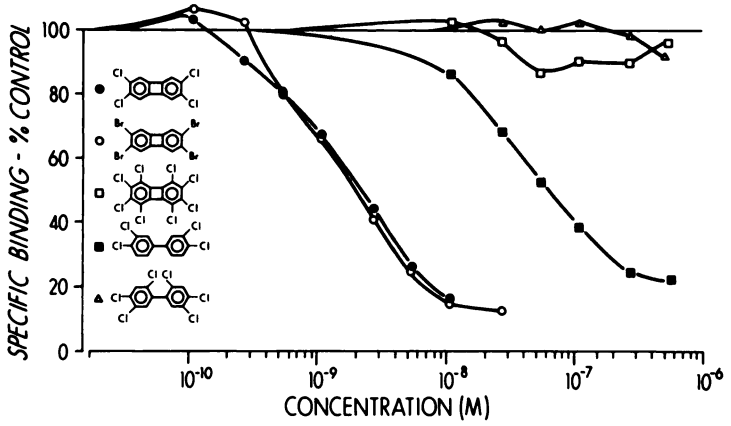


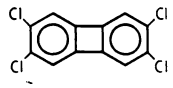
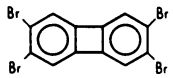
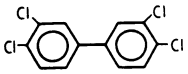
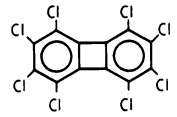
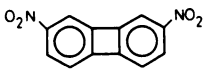
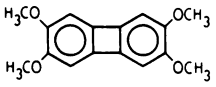
FIG. 2. *Competitive binding by biphenyl and biphenylene congeners for specific binding of [^{3}H]TCDD to hepatic cytosol*

The specific binding of [^{3}H]TCDD was measured as described in MATERIALS AND METHODS. The concentration of [^{3}H]TCDD was 0.54 nM, and the concentrations of unlabeled congeners are given on the abscissa.

TABLE 3

Biological potency and binding affinity of various biphenylene and biphenyl congeners

The biological potency (ED_{50}) for each compound was estimated from a log dose-response curve for the induction of hepatic aryl hydrocarbon hydroxylase activity in the chicken embryo, as described in the legend to Table 1. For inactive compounds, the highest dose tested is given in parentheses. The affinity of these compounds for the TCDD-binding species in hepatic cytosol of C57BL/6J mice was determined by their capacity to compete with the specific binding of [^{3}H]TCDD (see MATERIALS AND METHODS). For inactive compounds the highest concentration tested which was judged to be soluble is given in parentheses. The biological potency of TCDD is 0.2 nmole/kg, and its binding affinity is 0.27 nM (11).

	BIOLOGICAL POTENCY ED_{50} (n mol/kg)	BINDING AFFINITY K_D (nM)
	0.28	0.34
	0.44	0.47
	88	15
	INACTIVE (470)	INACTIVE (540)
	INACTIVE (930)	INACTIVE (540)
	INACTIVE (930)	INACTIVE (540)

phenylene and 2,3,6,7-tetrabromobiphenylene were roughly equipotent to TCDD (ED_{50} = 0.2 nmole/kg; K_D = 0.27 nM), and that 3,4,3′,4′-tetrachlorobiphenyl was considerably less potent as an inducer and less avid a binder *in vitro*. The other biphenylene analogues were inactive in inducing hepatic aryl hydrocarbon hydroxylase activity and failed to compete with [^{3}H]TCDD for hepatic cytosol binding sites. All the biphenyl congeners that failed to induce the hydroxylase activity (compounds *1–8* and *12–16* in Table 1 and compounds *17–20*) showed no affinity for the cytosol binding sites at concentrations of 540 nM or higher, 1000 times the concentration of the radioligand. In contrast, the three halogenated biphenyl congeners that induced the hydroxylase activity—3,4,3′,4′-tetrachlorobiphenyl (*9*), 3,4,5,3′,4′,5′-hexachlorobiphenyl (*10*), and 3,4,5,3′,4′,5′-hexabromobiphenyl (*11*)—all competed with [^{3}H]TCDD for specific cytosol binding sites. For compounds *10* and *11* we were unable to estimate binding affinities because the limited aqueous solubility of these congeners prevented us from reliably achieving a soluble concentration sufficient to compete for at least half the specific binding of [^{3}H]TCDD.

Induction of Hepatic Aryl Hydrocarbon Hydroxylase Activity in C57BL/6J and DBA/2J Mice

It is of considerable interest to examine the effects of 3,4,3′,4′-tetrachlorobiphenyl on hepatic microsomal monooxygenase activity in certain inbred strains of mice. The administration of polycyclic aromatic hydrocarbons, such as MC, induces hepatic aryl hydrocarbon hydroxylase activity and cytochrome P_1-450 in C57BL/6J mice, but not in DBA/2J mice. TCDD, which is 30,000 times as potent an inducer as MC, will induce hepatic aryl hydrocarbon hydroxylase activity in both strains of mice, but a larger dose is required in DBA/2J mice (28, 29). DBA/2J mice appear to have an altered receptor for enzyme induction, a receptor protein with a diminished affinity for inducing compounds (11). This leads to nearly complete unresponsiveness to MC and diminished sensitivity to the more potent inducer, TCDD.

The administration of 3,4,3′,4′-tetrachlorobiphenyl to C57BL/6J mice produced a dose-related induction of hepatic aryl hydrocarbon hydroxylase activity, with an ED_{50} of 20 μmoles/kg (5.8 mg/kg) (Fig. 3). 3,4,3′,4′-Tetrachlorobiphenyl was approximately 200 times less potent in the C57BL/6J mouse than in the chicken embryo (ED_{50} = 88 nmoles/kg). The cause of this relatively diminished potency in the

mouse is unknown, but it may be related to more rapid metabolic inactivation. In C57BL/6J mice, 3,4,3',4'-tetrachlorobiphenyl was still 3 times as potent an inducer as MC (ED_{50} = 59 μmoles/kg).

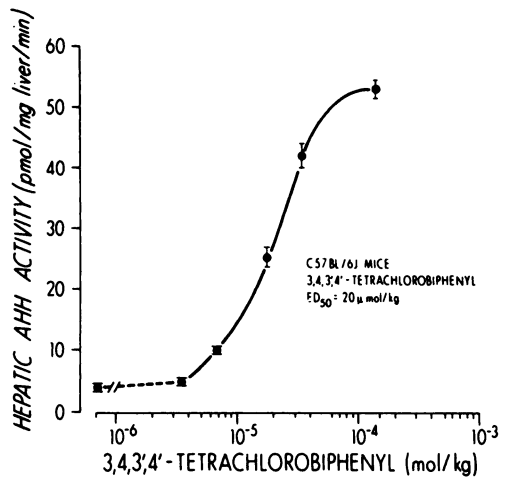


FIG. 3. *Log dose-response curve for induction of hepatic aryl hydrocarbon hydroxylase (AHH) activity in C57BL/6J mice by 3,4,3',4'-tetrachlorobiphenyl*

C57BL/6J mice, 7-week-old females, were given a single intraperitoneal injection of corn oil (controls) or various doses of 3,4,3',4'-tetrachlorobiphenyl dissolved in corn oil. Forty-eight hours later the animals were killed and their livers were assayed for aryl hydrocarbon hydroxylase activity.

Maximally inducing doses of 3,4,3',4'-tetrachlorobiphenyl and TCDD produced the same degree of induction of hepatic aryl hydrocarbon hydroxylase activity and cytochrome P_1-450 in C57BL/6J mice (Table 4). DBA/2J mice responded to TCDD in a comparable manner but failed to respond to 3,4,3',4'-tetrachlorobiphenyl, even at a very high dose (911 μmoles/kg). The dose of 3,4,3',4'-tetrachlorobiphenyl was 45 times the ED_{50} in C57BL/6J mice. This is similar to the pattern observed for MC. The ED_{50} for MC in C57BL/6J mice is 59 μmoles/kg, but administration of 50 times this amount (2.98 mmoles/kg) produces no effect in DBA/2J mice (28). It is possible that DBA/2J mice might respond to even higher doses of MC or 3,4,3',4'-tetrachlorobiphenyl, but they must be at least 250–500 times less sensitive than C57BL/6J mice. In contrast, DBA/2J mice are only 10 times less sensitive to TCDD than C57BL/6J mice ($ED_{50} \geqq$ 10 nmoles/kg in DBA/2J and 1 nmole/kg in C57BL/6J mice). TCDD, MC, and 3,4,3',4'-tetrachlorobiphenyl all appear to act on the same receptor. At present we are unable to explain why there is only a 10-fold difference in strain sensitivity to TCDD and a very large (or

TABLE 4

Effect of 3,4,3',4'-tetrachlorobiphenyl and TCDD administration on hepatic aryl hydrocarbon hydroxylase activity and cytochrome P-450 in C57BL/6J and DBA/2J mice

C57BL/6J mice, 8-week-old males, were injected intraperitoneally with corn oil, 8 ml/kg (controls); 3,4,3',4'-tetrachlorobiphenyl, 40 mg/kg dissolved in corn oil; or TCDD, 9.66 μg/kg dissolved in *p*-dioxane. DBA/2J mice, 9-week-old females, were injected with corn oil (controls); 3,4,3',4'-tetrachlorobiphenyl dissolved in corn oil (266 mg/kg given in two equally divided doses 24 hr apart), or TCDD, 96.6 μg/kg dissolved in *p*-dioxane. Forty-eight hours after administration, the animals were killed and their livers were assayed for aryl hydrocarbon hydroxylase activity and cytochrome P-450. Each value is the mean ± standard error for four or five animals.

Treatment	Dose	Aryl hydrocarbon hydroxylase activity	Cytochrome P-450	Absorption maximum[a]
	moles/kg	*pmoles/mg liver/min*	*nmoles/mg protein*	*nm*
C57BL/6J mice				
Control		5.66 ± 0.20	0.619 ± 0.009	450.2 ± 0.1
3,4,3',4'-Tetrachlorobiphenyl	1.4×10^{-4}	62.4 ± 3.8	1.853 ± 0.152	448.6 ± 0.1
TCDD	3.0×10^{-8}	60.7 ± 6.0	1.598 ± 0.105	448.3 ± 0.1
DBA/2J mice				
Control		3.20 ± 0.18	0.596 ± 0.005	450.3 ± 0.2
3,4,3',4'-Tetrachlorobiphenyl	9.1×10^{-4}	3.53 ± 0.19	0.722 ± 0.037	450.0 ± 0.1
TCDD	3.0×10^{-7}	76.2 ± 10.7	1.788 ± 0.169	448.4 ± 0.1

[a] Absorption maximum of the CO-reduced vs. reduced difference spectrum of microsomes.

infinite) strain difference for weaker inducers like MC or 3,4,3′,4′-tetrachlorobiphenyl.

We tested several other halogenated biphenyl congeners for their capacity to induce hepatic aryl hydrocarbon hydroxylase activity in C57BL/6J mice. The results (Table 5) correspond to the structure-activity relationship observed in the chicken embryo (Table 1). 3,4,3′,4′-Tetrachlorobiphenyl (*9*), 3,4,5,3′,4′,5′-hexachlorobiphenyl (*10*), and 3,4,5,3′,4′,5′-hexabromobiphenyl (*11*) induced hepatic aryl hydrocarbon hydroxylase activity to similar extents, whereas 2,4,5,2′,4′,5′-hexachlorobiphenyl (*13*) and 2,3,4,2′,3′,4′-hexachlorobiphenyl (*12*), even at 10-fold higher doses, produced little enzyme induction.

Effects of Halogenated Biphenyls on Microsomal Monooxygenase Activities in Rat Liver

From the data presented, it is clear that 3,4,3′,4′-tetrachlorobiphenyl (*9*), 3,4,5,3′,4′,5′-hexachlorobiphenyl (*10*), and 3,4,5,3′,4′,5′-hexabromobiphenyl (*11*) induce microsomal monooxygenase activity to an extent comparable to MC. We wished to determine whether these congeners are pure MC-like inducers, devoid of phenobarbital-like activity, and also to screen some of the other chlorobiphenyl congeners for their potential phenobarbital-like activity. Aminopyrine *N*-demethylase activity is induced by phenobarbital but is not significantly induced by MC or TCDD (19). Thus it would appear to be a good selective response for phenobarbital-like activity. In rats, both MC and phenobarbital induce hepatic aryl hydrocarbon hydroxylase activity, but induction by phenobarbital is much less and is not accompanied by a selective increase in cytochrome P_1-450 (5).

As shown in Table 6, the administration of phenobarbital to rats produced a modest increase in hepatic aryl hydrocarbon hydroxylase activity and a nearly 5-fold stimulation of aminopyrine *N*-demethylase ac-

TABLE 5

Effects of various halogenated biphenyls on hepatic aryl hydrocarbon hydroxylase activity in C57BL/6J mice

C57BL/6J immature female mice were injected with a single dose of corn oil (control), 8 mg/kg, or various halogenated biphenyls dissolved in corn oil. Forty-eight hours later the animals were killed and their livers were assayed for aryl hydrocarbon hydroxylase activity. Each value is the mean ± standard error for four mice.

Treatment	Dose	Activity
	moles/kg	*pmoles/mg liver/min*
Control		5.3 ± 0.1
3,4,3′,4′-Tetrachlorobiphenyl	3.42×10^{-5}	52.2 ± 2.8
3,4,3′,4′-Tetrachlorobiphenyl	1.71×10^{-4}	50.6 ± 4.0
3,4,5,3′,4′,5′-Hexachlorobiphenyl	2.77×10^{-5}	50.9 ± 3.5
3,4,5,3′,4′,5′-Hexabromobiphenyl	2.77×10^{-5}	60.1 ± 6.7
2,4,5,2′,4′,5′-Hexachlorobiphenyl	2.77×10^{-4}	7.7 ± 0.7
2,3,4,2′,3′,4′-Hexachlorobiphenyl	2.77×10^{-4}	8.2 ± 0.3

TABLE 6

Effects of phenobarbital, TCDD, 3,4,3′,4′-tetrachlorobiphenyl, and Aroclor 1254 on hepatic aryl hydrocarbon hydroxylase and aminopyrine N-demethylase activities in the rat

Male Sprague-Dawley rats (50–60 g) were injected with a single intraperitoneal dose of corn oil, 8 ml/kg (control); 3,4,3′,4′-tetrachlorobiphenyl, 100 mg/kg, in corn oil; Aroclor 1254, 500 mg/kg, in corn oil; or TCDD, 9.66 μg/kg, in *p*-dioxane (0.4 ml/kg), or were injected daily for 5 days with sodium phenobarbital, 75 mg/kg, in 0.9% NaCl. Five days after the initial injection the animals were killed and their livers were assayed for the enzyme activities as described in MATERIALS AND METHODS. Each value represents the mean ± standard error of determinations in five animals.

Treatment	Aryl hydrocarbon hydroxylase activity	Aminopyrine *N*-demethylase activity
	pmoles/mg liver/min	
Control	2.53 ± 0.18	90.0 ± 6.5
Phenobarbital	10.9 ± 0.9	434.4 ± 38.8
TCDD	36.5 ± 1.9	97.2 ± 4.7
3,4,3′,4′-Tetrachlorobiphenyl	36.8 ± 2.2	111.3 ± 7.9
3,4,3′,4′-Tetrachlorobiphenyl + TCDD	33.6 ± 2.7	90.7 ± 5.8
Aroclor 1254	47.4 ± 1.2	318.6 ± 15.3

tivity. In contrast, TCDD produced a much larger increase in hepatic hydroxylase activity and no significant change in *N*-demethylase activity. 3,4,3′,4′-Tetrachlorobiphenyl induced a pattern like that of TCDD, and the combined administration of maximally inducing doses of 3,4,3′,4′-tetrachlorobiphenyl and TCDD produced no greater effect than that evoked by either drug alone. In contrast, Aroclor 1254 produced a substantial increase in both enzyme activities. Aroclor 1254 induction of the hydroxylase activity was greater than that produced by TCDD, the response being comparable to the additive effects of phenobarbital and TCDD. *N*-Demethylase activity was substantially induced by Aroclor 1254 (3.5-fold), but to a lesser extent than by phenobarbital.

The microsomal cytochrome P-450 induced by phenobarbital had a CO absorption maximum at 450 nm, that induced by TCDD and 3,4,3′,4′-tetrachlorobiphenyl had maxima at approximately 448.1 nm, and Aroclor 1254-induced microsomes had an intermediate maximum at 448.6 nm.

We examined the capacity of other chlorinated biphenyl isomers to induce aryl hydrocarbon hydroxylase and aminopyrine *N*-demethylase activities in rat liver (Table 7). The results are expressed as the fractional increases in these enzyme activities compared with the activities in control animals. Phenobarbital produced a 4-fold increase in hydroxylase activity and a 4.5-fold increase in *N*-demethylase activity (similar to that shown in Table 6). The three hexachlorobiphenyls all produced small increases in the hydroxylase activity (comparable to that evoked by phenobarbital) and moderate increases in aminopyrine *N*-demethylase activity. The 2,4,5,-2′,4′,5′-hexachloro congener (*13*) produced a 3.7-fold increase in *N*-demethylase activity, 2,3,4,2′,3′,4′-hexachlorobiphenyl (*12*) produced a 2.2-fold increase, and 2,4,6,2′,4′,6′,-hexachlorobiphenyl (*14*) was the least active (1.9-fold increase). These three nonplanar hexachlorobiphenyls appear to induce a phenobarbital-like response. Decachlorobiphenyl caused a small increase in both enzyme activities, which is of questionable significance. 3,4,5,3′,-4′,5′-Hexabromobiphenyl markedly stimulated hepatic aryl hydrocarbon hydroxylase activity. This congener produced a small increase in *N*-demethylase activity, comparable to that produced by decachlorobiphenyl, and of questionable significance.

TABLE 7

Effects of halogenated biphenyl congeners on hepatic aryl hydrocarbon hydroxylase and aminopyrine N-demethylase activities in rats

Male Sprague-Dawley rats (50–70 g) were injected with corn oil (8 ml/kg) or 2,4,5,2′,4′,5′-hexachlorobiphenyl (50 mg/kg), 2,3,4,2′,3′,4′-hexachlorobiphenyl (50 mg/kg), 2,4,6,2′,4′,6′-hexachlorobiphenyl (50 mg/kg), or decachlorobiphenyl (50 mg/kg), all dissolved in corn oil, on days 1, 3, and 5. 3,4,5,3′,4′,5′-Hexabromobiphenyl (20 mg/kg) dissolved in corn oil was administered on days 1 and 4; sodium phenobarbital (75 mg/kg) dissolved in 0.9% NaCl was administered for 5 days. On day 6 the animals were killed and their hepatic enzyme activities were analyzed as described in MATERIALS AND METHODS. The results are expressed as the mean of enzyme activities in the treated groups relative to the mean of the activities in the control group (corn oil). The absolute activities in the control group (mean ± standard error) were: aryl hydrocarbon hydroxylase activity, 1.64 ± 0.14 pmoles of product per milligram of liver per minute; aminopyrine *N*-demethylase activity, 33.75 ± 0.94 pmoles of product per milligram of liver per minute. Each group consisted of four or five animals. The standard error in each group was generally less than 10% of the mean value.

Treatment	Total dose	Aryl hydrocarbon hydroxylase activity	Aminopyrine *N*-demethylase activity
	mmoles/kg		
Control		1.00	1.00
Phenobarbital	1.48	4.12	4.55
2,4,5,2′,4′,5′-Hexachlorobiphenyl	0.42	3.64	3.71
2,3,4,2′,3′,4′-Hexachlorobiphenyl	0.42	3.70	2.22
2,4,6,2′,4′,6′-Hexachlorobiphenyl	0.42	2.32	1.87
Decachlorobiphenyl	0.30	1.47	1.43
3,4,5,3′,4′,5′-Hexachlorobiphenyl	0.06	16.07	1.51

DISCUSSION

In this report we have examined a series of halogenated biphenyls for their potency

to induce hepatic aryl hydrocarbon hydroxylase activity and cytochrome P_1-450. Only three congeners, 3,4,3′,4′-tetrachlorobiphenyl (*9*), 3,4,5,3′,4′,5′-hexachlorobiphenyl (*10*), and 3,4,5,3′,4′,5′-hexabromobiphenyl (*11*), induced hepatic aryl hydrocarbon hydroxylase activity in the chicken embryo. These three congeners also induced the hydroxylase activity in mouse and rat liver and bound to the hepatic cytosol protein, which appears to be the receptor site for the induction of cytochrome P_1-450. These three halobiphenyls appear to be MC-like inducers, devoid of significant phenobarbital-like activity. Among the chlorinated biphenyl congeners which failed to induce hepatic aryl hydrocarbon hydroxylase activity, a few compounds did stimulate hepatic aminopyrine *N*-demethylase in the rat.

Our results suggest that while the commercial mixture of chlorobiphenyl isomers, Aroclor 1254, induces a pattern of microsomal monooxygenase activities resembling that seen with the combined administration of MC and phenobarbital, any given chlorobiphenyl congener produces either an MC-like response or a phenobarbital-like response or is devoid of both activities. While the data suggest that any given congener is capable of evoking only one of these two responses, we cannot make a categorical statement because (a) we tested only a few of the 209 possible chlorobiphenyl congeners, and (b) if a congener could elicit both activities but differed greatly in its potency to evoke them, we would probably detect only the response for which it had the greater potency.

The many compounds that induce cytochrome P_1-450 and aryl hydrocarbon hydroxylase activity can be divided into two groups, the polycyclic aromatic hydrocarbons and the halogenated aromatic compounds (dibenzo-*p*-dioxins, dibenzofurans, and azoxybenzenes). All these compounds are lipophilic, all are planar, and all (that we have tested) bind to the hepatic cytosol protein, which appears to be the receptor for induction of cytochrome P_1-450. For the polycyclic aromatic hydrocarbons, it is not possible at this time to discern the structural features necessary for receptor binding. In contrast, for the halogenated aromatic inducers one finds a well-defined structure-activity relationship (11, 25, 27). Consider 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran, and 3,4,3′,4′-tetrachloroazoxybenzene as prototypes of their respective groups. These compounds are approximate isostereomers, their molecular structures can be thought of as fitting into a rectangle approximately 3 × 10 A, with halogen atoms in all four corners of the rectangle. Among the halogenated dibenzo-*p*-dioxins (the best studied of the groups), halogen atoms must occupy at least three of the four lateral ring positions (2, 3, 7, and 8), and the order of potency of halogen substitution is Br > Cl > F (25, 30).

We initiated this study of the halogenated biphenyls because of the obvious structural similarity of certain of the congeners to the halogenated aromatic compounds which induce cytochrome P_1-450. 3,4,3′,4′-Tetrachlorobiphenyl (*9*) is an approximate stereoisomer of TCDD (Fig. 1), and 3,4,5,3′,4′,5′-hexahalobiphenyls (*10* and *11*) are roughly isosteric with two potent hexachlorodibenzo-*p*-dioxins, the 1,2,3,6,7,8-hexachloro and 1,2,3,7,8,9-hexachloro congeners. As we have shown, these three chlorobiphenyls (compounds *9*, *10*, and *11*) do induce cytochrome P_1-450 and aryl hydrocarbon hydroxylase activity and do bind to the hepatic cytosol binding protein. Certain other chlorobiphenyl congeners that were not commercially available, such as 3,4,3′-trichlorobiphenyl, 3,4,4′-trichlorobiphenyl, and 3,4,5,3′,4′-pentachlorobiphenyl, would seem likely to have biological activity.

The chlorinated biphenyls that have chlorine atoms substituted in positions 2, 2′, 6, or 6′ did not induce aryl hydrocarbon hydroxylase activity (Table 1) and did not compete for the hepatic cytosol binding sites. Such substitutions adjacent to the biphenyl bridge produce marked nonplanarity of the benzene rings. All compounds that induce cytochrome P_1-450 appear to be planar or very nearly so.

Even for the biologically active halogenated biphenyls (compounds *9*, *10*, and *11*),

the preferred configuration is not completely planar. 3,4,3′,4′-Tetrachlorobiphenyl is less planar than TCDD, 2,3,7,8-tetrachlorodibenzofuran, and 3,4,3′,4′-tetrachloroazoxybenzene, and its biological potency is 100-fold less. Converting 3,4,3′,4′-tetrachlorobiphenyl to 2,3,6,7-tetrachlorobiphenylene (Fig. 1), we construct a rigid, absolutely planar compound, which is over 100 times more potent. The importance of the halogenated biphenylenes is twofold: they support the proposed structure-activity relationship for chlorinated biphenyls, and they represent a new class of very potent inducers of cytochrome P_1-450.

It is obvious that the current scientific interest in all the halogenated aromatic compounds is not purely academic, but arises from their toxicity and their dispersion in our environment. While it is beyond the scope of this report to review the toxicology of these compounds, it is useful to comment on their toxic potency in the light of the structure-activity relationship for the induction of aryl hydrocarbon hydroxylase activity. (For an excellent review of the toxicology of these compounds, see ref. 31.) For the halogenated dibenzo-*p*-dioxins and dibenzofurans there is a very good correlation between the toxic potency of a congener (its LD_{50} or potency to produce chloracne) and its potency to induce hepatic aryl hydrocarbon hydroxylase activity (25). Studies on a more limited series of chlorinated azoxybenzenes indicate a good correlation between the capacity of a congener to produce chloracne and its potency to induce aryl hydrocarbon hydroxylase activity (27). Information on the comparative toxicity of various chlorobiphenyl congeners is limited, but one report by McKinney *et al.* (32) is most suggestive. These authors compared the toxicological effects in chickens of five hexachlorobiphenyl congeners: 2,3,6,2′,3′,6′-hexachloro-, 2,4,6,2′,4′,6′-hexachloro-, 2,3,4,2′,3′,4′-hexachloro-, 2,4,5,2′,4′,5′-hexachloro-, and 3,4,5,3′,4′,5′-hexachlorobiphenyl. 3,4,5,3′,4′,5′-Hexachlorobiphenyl had the greatest toxic potency of these congeners and was the only one to produce significant edema and thymic atrophy, toxic responses produced by TCDD and 2,3,7,8-tetrachlorodibenzofuran.

For several classes of halogenated aromatic compounds—dibenzo-*p*-dioxins, dibenzofuran, axozybenzenes, and biphenyls—there is a rather good correlation between the capacity of a congener to bind to hepatic cytosol binding species (and induce hepatic aryl hydrocarbon hydroxylase activity) and its capacity to produce certain toxic responses (chloracne, thymic atrophy, and edema in chickens). Genetic evidence suggests not only that the hepatic cytosol protein is the receptor for the induction of cytochrome P_1-450, but that the receptor controls the expression of a number of genes (33). We do not believe that the induction of aryl hydrocarbon hydroxylase activity per se results in toxicity, but rather suggest that toxic syndromes we observe may be mediated by one (or more) of the other members of this gene battery which are coordinately expressed by halogenated aromatic compounds.

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