Biochemical Pharmacology, Vol. 58, pp. 29–38, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00070-2

Inductive and Inhibitory Effects of Non-*ortho*-substituted Polychlorinated Biphenyls on Estrogen Metabolism and Human Cytochromes P450 1A1 and 1B1

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ABSTRACT. The effects of a series of non-*ortho*-substituted polychlorinated biphenyls (PCBs) on human cytochrome P450 1A1 (CYP1A1), a 17β-estradiol (E_2) 2-hydroxylase, and P450 1B1 (CYP1B1), an E_2 4-hydroxylase, were investigated in HepG2 and MCF-7 cells. Elevated rates of 2- and 4-methoxyestradiol (2- and 4-$MeOE_2$) formation in PCB-treated cultures were measured as activities of CYP1A1 and CYP1B1, respectively. Of the congeners investigated, 3,4,4′,5-tetrachlorobiphenyl (PCB 81), 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), and 3,4′,5-trichlorobiphenyl (PCB 39) caused marked stimulation of E_2 metabolism in both cell lines. Northern blot analyses confirmed that exposure of MCF-7 cells to PCBs 81, 126, and 39 caused highly elevated levels of the CYP1A1 and CYP1B1 mRNAs. Exposure of MCF-7 cells to 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 169) resulted in elevated levels of the CYP1A1 and CYP1B1 mRNAs, but did not cause elevated rates of E_2 metabolism; rather, 4-$MeOE_2$ production was depressed to below control levels in PCB 169-treated cultures. PCB 169 also inhibited the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced 4-$MeOE_2$ and, to a lesser extent, 2-$MeOE_2$ production in MCF-7 cells, as did PCB 126 and several other congeners. In microsomal assays, inhibition of cDNA-expressed human CYP1B1 by PCBs 169 and 126 was demonstrated. These studies with one subgroup of PCBs, the non-*ortho*-substituted congeners, underscore the complexity and diversity of effects of PCBs, as individual congeners were found both to induce expression and to inhibit activity of human CYP1B1 and CYP1A1. BIOCHEM PHARMACOL **58**;1:29–38, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. polychlorinated biphenyls; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; cytochrome P450; CYP1A1; CYP1B1; estrogen metabolism

PCBs** are widespread environmental pollutants that elicit a broad spectrum of toxic effects in mammals and other vertebrate species [1–3]. While individual congeners differ markedly in their chemical and toxicologic properties, certain classes of PCB congeners are thought to have common mechanisms of action with regard to their toxicity. For the non-*ortho*-substituted PCB congeners, toxicity is thought to be mediated primarily by interactions with the AhR, a ligand-activated, basic helix-loop-helix transcription factor that binds certain planar molecules including polycyclic aromatic hydrocarbons and TCDD with high affinity [4]. The low energy barrier for attainment of a planar configuration [5] allows some non-*ortho*-substituted PCB congeners to bind to and activate the AhR. The ligand-bound AhR potentiates transcription of a number of genes, referred to as the Ah gene battery [6], which includes those encoding cytochromes P450 of the *CYP1* gene family, several UDP-glucuronosyltransferases, NAD(P)H:quinone oxidoreductase, and aldehyde dehydrogenase. In some instances, however, the ligand-bound AhR appears to suppress rather than enhance specific gene transcription [7].

TCDD is the ligand with the highest known affinity for the AhR, and is the most potent compound identified in eliciting AhR-mediated responses. To evaluate and quantify the TCDD-like toxic effects of related compounds, the

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** *Abbreviations:* PCB, polychlorinated biphenyl; AhR, aromatic hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; P450 or CYP, cytochrome P450; TEF, toxic equivalency factor; E_2, 17β-estradiol; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; $MeOE_2$, methoxyestradiol; GAPDH, glyceraldehyde phosphate dehydrogenase; IEF, inductive equivalency factor; AHH, aryl hydrocarbon hydroxylase; and EROD, ethoxyresorufin *O*-deethylase.

Received 15 July 1998; accepted 3 November 1998.

toxic equivalency system was devised whereby *in vivo* and *in vitro* effects elicited by a compound are compared with those of TCDD in the same system [8–11]. TEF values have been assigned to a number of polyhalogenated dioxin, furan, and biphenyl congeners, and these are used in mechanism-based risk assessments for estimating toxicity to humans. A possible confounding factor in the use of this system is that the database used for the establishment of the TEF values for the most part originated from determinations with rodents and rodent-derived cell lines in culture. While the relative potencies of compounds in rodents *in vivo* often correlate well with determinations with rodent-derived cells *in vitro*, the results of recent studies suggest that there may be significant differences in the relative potencies of halogenated aromatic hydrocarbons in human as compared with rodent cells [12], possibly related to differences in the binding affinities of these compounds for the human versus rodent AhR.

In studies to evaluate the effects of a series of non-*ortho*-substituted PCB congeners on human AhR-regulated gene expression, we used two human-derived cell lines: HepG2, derived from a hepatocarcinoma, and MCF-7, derived from a mammary adenocarcinoma. We evaluated the effects of these compounds on expression of two enzymes of the human Ah gene battery, CYP1A1, an E_2 2-hydroxylase [13], and CYP1B1, an E_2 4-hydroxylase [14, 15]. In HepG2 cells, CYP1A1, but not CYP1B1, is induced by TCDD, whereas in MCF-7 both CYP1A1 and CYP1B1 are induced by TCDD [14]. The results of these studies have been published in abstract form [16, 17].

MATERIALS AND METHODS

Chemicals

TCDD was purchased from Cambridge Isotope Laboratories. The following PCB congeners (99+% purity) were obtained from AccuStandard: PCBs 9 (2,5-dichlorobiphenyl), 10 (2,6-dichlorobiphenyl), 28 (2,4,4′-trichlorobiphenyl), 29 (2,4,5-tetrachlorobiphenyl), 30 (2,4,6-trichlorobiphenyl), 35 (3,3′,4-trichlorobiphenyl), 36 (3,3′,5-trichlorobiphenyl), 37 (3,4,4′-trichlorobiphenyl), 39 (3,4′,5-trichlorobiphenyl), 54 (2,2′,6,6′-tetrachlorobiphenyl), 77 (3,3′,4,4′-tetrachlorobiphenyl), 78 (3,3′,4,5-tetrachlorobiphenyl), 79 (3,3′,4,5′-tetrachlorobiphenyl), 80 (3,3′,5,5′-tetrachlorobiphenyl), 81 (3,4,4′,5-tetrachlorobiphenyl), 118 (2,3′,4,4′,5-pentachlorobiphenyl), 126 (3,3′,4,4′,5-pentachlorobiphenyl), 127 (3,3′,4,5,5′-pentachlorobiphenyl), 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), and 169 (3,3′,4,4′,5,5′-hexachlorobiphenyl). Estrogen metabolite standards were from Steraloids. BSTFA was from Pierce. Type H-2 β-glucuronidase/sulfatase was from the Sigma Chemical Co.

Culture of HepG2 and MCF-7 Cells

Cells were cultured in 75-cm^2 flasks or 6-well plates (2-cm^2 wells). The medium for propagation of HepG2 cells consisted of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin [14]. For culture of MCF-7 cells, Dulbecco's modified Eagle's medium was supplemented with 5% bovine calf serum, nonessential amino acids, 10 ng/mL of insulin, 100 U/mL of penicillin, and 100 μg/mL of streptomycin as previously described [18]. Cultures were maintained at 37° in a humidified atmosphere with 5% CO_2 in air.

Effects of PCB Exposure on Cell Viability

Cultures of HepG2 and MCF-7 cells were exposed to PCB congeners at a 1 μM concentration for 72 hr. The effects of this exposure on cell viability were determined by trypan blue dye exclusion [19], and cells were counted with a hemocytometer.

Effects of PCB Congeners on E_2 Metabolism

To determine the effects of pretreatment with PCBs on E_2 metabolism in the two cell lines, cultures were exposed to concentrations of PCB congeners ranging from 10 pM to 1 μM, or to 10 nM TCDD. Control cultures received the solvent vehicle only, DMSO at 0.1% (v/v) in medium. After 72 hr, medium was removed and replaced with medium containing 1 μM E_2 for 6 hr. This medium was then recovered for analysis of E_2 metabolites. Two-milliliter portions of medium were treated with β-glucuronidase/sulfatase as described [14] to hydrolyze conjugates. The estrogen metabolites were recovered by solid-phase extraction on Extrelut QE columns (EM Science), trimethylsilyl derivatives were prepared by reaction with BSTFA containing 10% (v/v) pyridine, and the derivatives were analyzed by GC/MS with stable isotope dilution [18, 20]. Metabolic rates were normalized to cellular protein content determined by the method of Bradford [21] using bovine serum albumin as the standard.

In experiments to investigate the antagonism of the TCDD-induced increase in E_2 metabolism by PCB congeners, cells were exposed to medium containing 10 nM TCDD alone or 10 nM TCDD plus 1 μM of a selected PCB congener. After 72 hr, medium was removed and replaced with medium containing 1 μM E_2 for 6 hr, and the production of 2- and 4-$MeOE_2$ was then determined as described above. In the experiments investigating inhibition of the TCDD-induced increase in E_2 metabolism, cells were exposed to 10 nM TCDD for 72 hr followed by a 6-hr exposure to 1 μM E_2 together with 1 μM of the PCB congener; then production of 2- and 4-$MeOE_2$ was determined.

Effects of Exposure to PCBs on CYP1A1 and CYP1B1 mRNA Levels

Total RNA was isolated from MCF-7 and HepG2 cell cultures by the guanidinium thiocyanate-phenol-chloroform extraction technique of Chomczynski and Sacchi [22].

RNA was treated with glyoxal [23] prior to electrophoresis in 1% (w/v) agarose gels. The RNA was transferred to Nytran membranes (Schleicher & Schuell), and blots were probed with CYP1A1 [24] or CYP1B1 [25, 26] and GAPDH [27] cDNAs that had been ^{32}P-labeled by random priming. The hybridizations were visualized by autoradiography and quantified by scanning densitometry.

E_2 Hydroxylation Catalyzed by cDNA-Expressed Human CYP1B1

Microsomal E_2 hydroxylation assays were performed essentially as previously described [13, 18]. The microsomal incubations contained 5 μM E_2, 35 μg of *Saccharomyces cerevisiae* microsomal protein containing cDNA-expressed CYP1B1 [15], 2 mM ascorbic acid, 5 mM $MgCl_2$, and 1.4 mM NADPH; they were buffered at pH 7.4 with 0.1 M sodium phosphate in a total volume of 250 μL. To investigate the inhibitory effects of PCBs on human CYP1B1, various concentrations of PCB congeners were preincubated with the microsomal reaction mixtures for 5 min at 37°, and the reactions were initiated by addition of the NADPH. Following incubation for an additional 10 min, the reactions were terminated by the addition of 2 vol. of ice-cold 30 mM ascorbic acid, the reaction mixtures were extracted with ethyl acetate, and metabolite-trimethylsilyl derivatives were prepared and analyzed by GC/MS.

Statistical Analysis

Data for the rates of E_2 metabolism in HepG2 and MCF-7 cultures for each of the treatment groups were subjected to one-way ANOVA and Dunnett's test for multiple comparisons to determine the statistical significance relative to solvent vehicle [0.1% (v/v) DMSO] controls. For determination of EC_{50} and IC_{50} values, concentration–response data were fit to the four-parameter sigmoidal function, $y = y_0 + a\{1 + e^{-[(x-x_o)/b]}\}^{-1}$, by using the SigmaPlot® program.

RESULTS

Effects of PCB Exposure on Viability of HepG2 and MCF-7 Cells

Exposure to each of the PCB congeners at 1 μM or to TCDD at 10 nM in medium for 72 hr had no significant effect on cell viability of HepG2 or MCF-7 cells as evaluated by trypan blue dye exclusion. After a 72-hr exposure to the solvent vehicle, 0.1% (v/v) DMSO in medium, 89.4 ± 4.2% (N = 3) of HepG2 cells totally excluded the dye. HepG2 cultures exposed to the PCB congeners at 1 μM or TCDD at 10 nM showed dye exclusion by more than 85% of the cells (means of N = 3). Similarly, after a 72-hr exposure to the DMSO solvent vehicle, 81.1 ± 4.5% (N = 3) of MCF-7 cells totally excluded the dye, while MCF-7 cultures exposed to each of the PCB congeners at 1 μM or TCDD at 10 nM showed dye exclusion by more than 79% of the cells (means of N = 3).

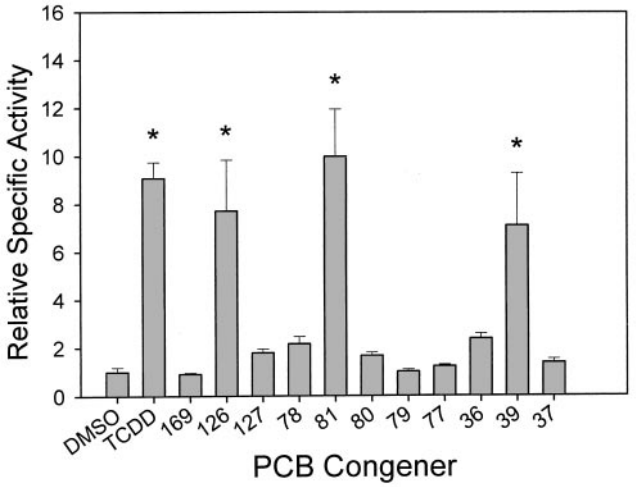

FIG. 1. Effects of TCDD and non-*ortho*-substituted PCB congeners on 2-$MeOE_2$ production in HepG2 cells. Cultures were exposed to the solvent vehicle (0.1% DMSO) as control, to 10 nM TCDD, or to 1 μM of the indicated PCB congener for 72 hr. Then medium was replaced with medium containing 1 μM E_2 for determination of 2-$MeOE_2$ production as described in Materials and Methods. Rates of 2-$MeOE_2$ formation are expressed relative to the control activity; data represent the means ± SEM from three experiments. The rate of 2-$MeOE_2$ formation in the control cultures was 3.08 ± 0.58 pmol(hr)$^{-1}$(mg)$^{-1}$ cellular protein. Key: (*) significant difference from DMSO control ($P < 0.05$).

Inductive Effects of PCB Exposure on Methoxyestrogen Formation in HepG2 and MCF-7 Cells

The effects of exposure to the non-*ortho*-substituted PCB congeners and to TCDD on 2-$MeOE_2$ formation in HepG2 cells are shown in Fig. 1. Exposure to 10 nM TCDD caused a 9-fold increase in the rate of 2-$MeOE_2$ formation. PCBs 126, 81, and 39 at 1 μM also caused statistically significant increases in 2-$MeOE_2$ formation, while the other non-*ortho*-substituted PCBs tested at 1 μM, including 77 and 169, did not. The concentration–response relationships for enhancement of 2-$MeOE_2$ formation by these congeners are shown in Fig. 2. The EC_{50} values for stimulation of 2-$MeOE_2$ formation were 0.57, 25, and 52 nM for TCDD and PCBs 126 and 81, respectively. The stimulation of 2-$MeOE_2$ formation by PCB 39 was not maximal over the concentration range tested.

The effects of exposure to the non-*ortho*-substituted PCB congeners at 1 μM and to TCDD at 10 nM on 2- and 4-$MeOE_2$ formation in MCF-7 cells are shown in Fig. 3. Similar to their effects in HepG2 cells, PCBs 126, 81, and 39 caused significant enhancement of 2-$MeOE_2$ (Fig. 3A) and 4-$MeOE_2$ (Fig. 3B) formation. PCB 78 also caused significant induction of both 2- and 4-$MeOE_2$ production, whereas it had no significant effect on 2-$MeOE_2$ production in HepG2 cells (Fig. 1). PCB 77 and several other non-*ortho*-substituted PCBs did not cause significant stimulation

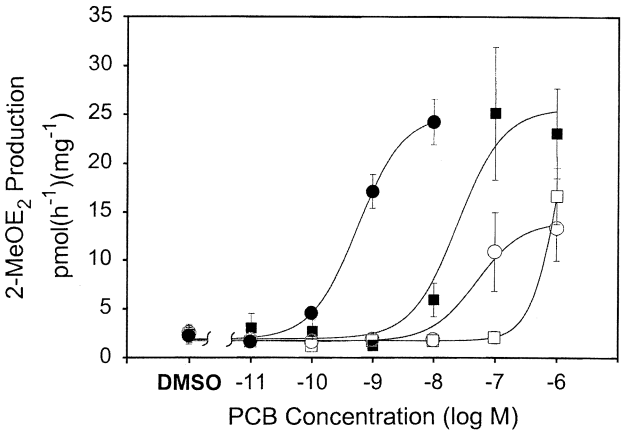

FIG. 2. Concentration–response curves for the effects of TCDD and PCBs 126, 81, and 39 on 2-MeOE$_2$ in HepG2 cells. Cultures were exposed to the solvent vehicle (0.1% DMSO) or to the indicated concentration of (●) TCDD, (■) PCB 81, (○) PCB 126, or (□) PCB 39 for 72 hr. Then medium was replaced with medium containing 1 μM E$_2$ for determination of 2-MeOE$_2$ production as described in Materials and Methods; data are the means ± SEM of determinations from three cultures.

of either 2-MeOE$_2$ or 4-MeOE$_2$ formation. PCB 169 had no significant effect on 2-MeOE$_2$ production, but caused a suppression rather than a stimulation of 4-MeOE$_2$ production. A series of *ortho*-substituted PCB congeners also was evaluated for the capacity to stimulate 2- and 4-MeOE$_2$ production in HepG2 and MCF-7 cells. When tested at 1 μM in the medium, PCBs 9, 10, 28, 29, 30, 54, 118, and 153 failed to induce significantly 2- or 4-MeOE$_2$ production in HepG2 or MCF-7 cells (data not shown).

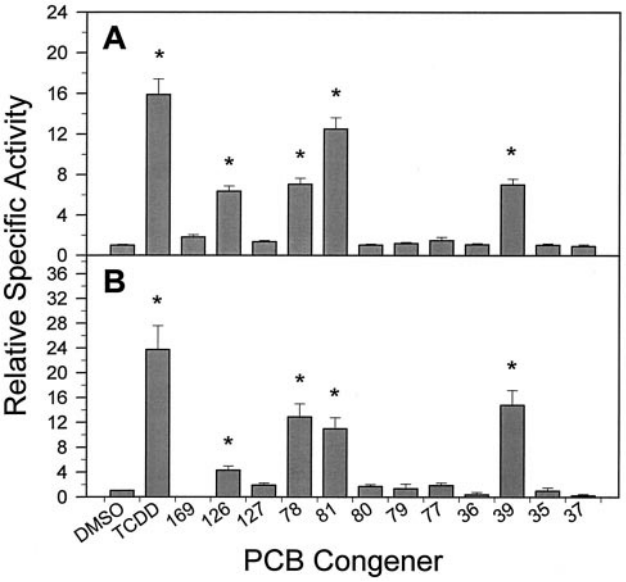

FIG. 3. Effects of TCDD and non-*ortho*-substituted PCB congeners on 2- and 4-MeOE$_2$ production in MCF-7 cells. Cultures were exposed to the solvent vehicle (0.1% DMSO), to 10 nM TCDD, or to 1 μM of the indicated PCB congener for 72 hr. Then medium was replaced with medium containing 1 μM E$_2$ for determination of 2- and 4-MeOE$_2$ production as described in Materials and Methods. Rates of 2-MeOE$_2$ (A) and 4-MeOE$_2$ (B) formation are expressed relative to the DMSO control; data are the means ± SEM of determinations from three cultures. Control activities were 0.318 ± 0.014 and 0.165 ± 0.014 pmol(hr)$^{-1}$(mg)$^{-1}$ of cellular protein for 2- and 4-MeOE$_2$ formation, respectively. Key: (*) significant difference from DMSO control ($P < 0.05$).

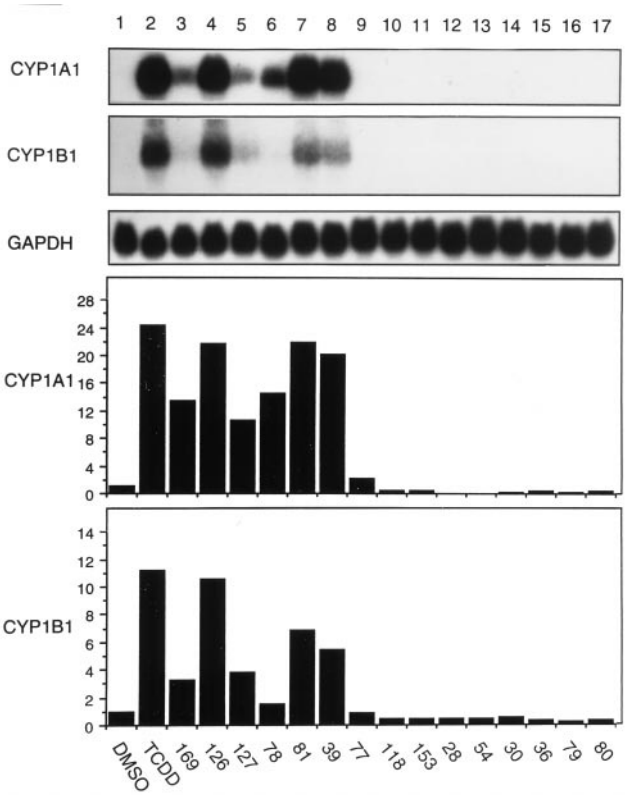

FIG. 4. Effects of TCDD and PCB congeners on CYP1A1 and CYP1B1 mRNA levels in MCF-7 cells. Cultures were exposed to the solvent vehicle (0.1% DMSO), to 10 nM TCDD, or to 1 μM of the indicated PCB congener for 72 hr. Total RNA was then isolated and subjected to northern analysis. Blots were probed with ^{32}P-labeled CYP1A1, CYP1B1, and GAPDH cDNAs as indicated, and quantified as described in Materials and Methods. The CYP1A1 and CYP1B1 mRNA levels, expressed relative to GAPDH mRNA, are shown in the lower panels.

Effects of PCB Exposure on CYP1A1 and CYP1B1 mRNA Levels in MCF-7 Cells

The effects of exposure to TCDD at 10 nM and a series of PCB congeners at 1 μM on CYP1A1 and CYP1B1 mRNA levels are shown in Fig. 4. As was observed previously [14, 28], and is shown here as a positive control, exposure to TCDD caused a marked elevation of both CYP1A1 and CYP1B1 mRNA levels in MCF-7 cells. Consistent with the effects on E$_2$ metabolism, exposure to PCBs 39, 81, and 126 caused the highest elevation of both CYP1A1 and CYP1B1 mRNA levels. PCBs 78, 127, and 169 also caused some induction of both mRNAs, whereas PCB 36, 77, 79, and 80 had minimal effects on the levels of the mRNAs. None of the *ortho*-substituted congeners tested, PCBs 28, 30, 54,

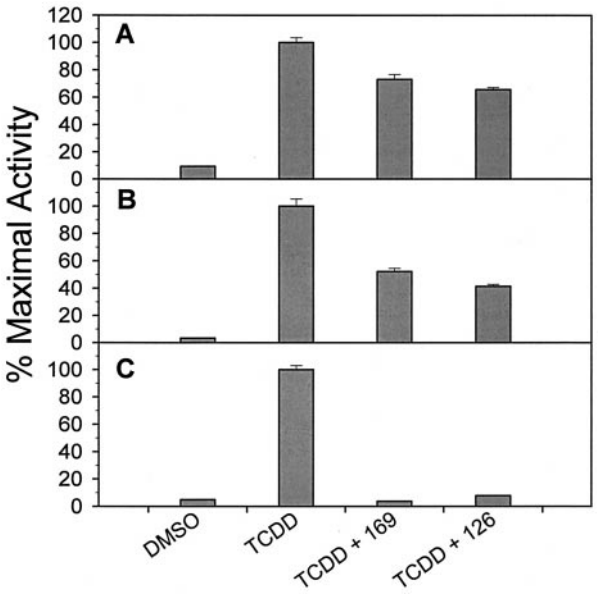

FIG. 5. Antagonistic effects of PCBs 169 and 126 on 2- and 4-$MeOE_2$ production in HepG2 and MCF-7 cells. Cultures of HepG2 (panel A) and MCF-7 cells (panels B and C) were exposed to the solvent vehicle (0.1% DMSO), 10 nM TCDD, 10 nM TCDD plus 1 μM PCB 169, or 10 nM TCDD plus 1 μM PCB 126. After 72 hr, medium was replaced with medium containing 1 μM E_2 for determination of 2-$MeOE_2$ (panels A and B) and 4-$MeOE_2$ formation (panel C). Rates of metabolite formation were normalized to cellular protein content and are expressed relative to the cultures receiving 10 nM TCDD alone; data are the means ± SEM of determinations from three cultures. Maximal rates of metabolism (TCDD-induced) were 8.57 ± 0.22, 23.1 ± 1.1, and 13.3 ± 1.1 $pmol(hr)^{-1}(mg)^{-1}$ in panels A, B, and C, respectively.

118, and 153, caused an elevation of CYP1A1 or CYP1B1 mRNA.

Inhibitory Effects of PCB Exposure on Methoxyestrogen Formation in HepG2 and MCF-7 Cells

The observation in MCF-7 cells that exposure to 1 μM PCB 169 caused a suppression rather than an elevation of 4-$MeOE_2$ production (Fig. 3), a measure of CYP1B1 activity [14, 15, 29], yet caused an elevated level of CYP1B1 mRNA (Fig. 4), prompted further investigation of the effects of PCB 169 on E_2 metabolism in the human cell lines. The effects of PCB126 were also investigated further, as the modest induction of 4-$MeOE_2$ production elicited by PCB 126 (Fig. 3B) did not appear consistent with the very high level of CYP1B1 mRNA induced by the congener (Fig. 4). To investigate possible antagonistic effects, HepG2 and MCF-7 cells were co-treated with 10 nM TCDD and 1 μM of either PCB 169 or PCB 126 for 72 hr prior to assay of E_2 metabolism. Both PCBs 169 and 126 antagonized the TCDD-induced production of 2-$MeOE_2$ in HepG2 cells, to 69 and 62% of the TCDD-induced level (Fig. 5A), respectively. Antagonistic effects of PCBs 169 and 126 on the TCDD-induced increase in E_2 metabolism were much more pronounced in the MCF-7 cell line. The formation of 2-$MeOE_2$ was depressed to 50 and 38% of the TCDD-induced level (Fig. 5B), and 4-$MeOE_2$ production was depressed to 5 and 8% of the TCDD-induced level (Fig. 5C) by co-treatment with TCDD and PCBs 169 and 126, respectively.

A possible mechanism for the apparent lack of or lower than expected induction of 4-$MeOE_2$ production and the potent antagonism of the TCDD-induced production of 4-$MeOE_2$ by PCBs 126 and 169 is that CYP1B1 is induced, but the enzyme is inhibited by PCBs 169 and 126. The smaller effects on 2-$MeOE_2$ production may be due to less effective inhibition of CYP1A1. To investigate this possibility, MCF-7 cells were treated with 10 nM TCDD for 72 hr to produce maximal induction of CYP1A1 and CYP1B1 and maximal rates of 2- and 4-$MeOE_2$ formation. Then various levels of PCBs 169 and 126 were added together with E_2 during the metabolism assay period. Under these conditions, both PCBs 169 and 126 caused concentration-dependent decreases in 2- and 4-$MeOE_2$ formation (Fig. 6). TCDD-induced 4-$MeOE_2$ production was inhibited more

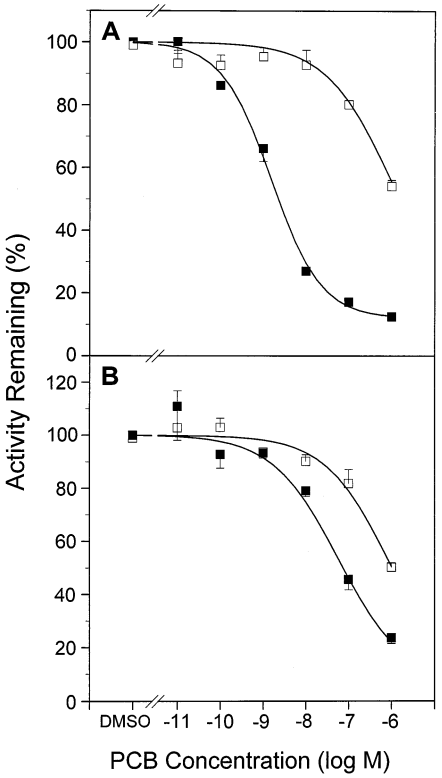

FIG. 6. Inhibition of the TCDD-induced 2- and 4-$MeOE_2$ production by PCBs 169 and 126 in MCF-7 cells. Cultures were exposed to 10 nM TCDD for 72 hr; then medium was replaced with medium containing 1 μM E_2 and the solvent vehicle (0.1% DMSO) or the indicated concentration of PCB 169 (panel A) or PCB 126 (panel B) for the determination of 2-$MeOE_2$ (□) and 4-$MeOE_2$ (■) formation. Rates of metabolite formation are expressed relative to the DMSO control; data are the means ± SEM of determinations from three cultures. Rates of TCDD-induced metabolism in the absence of added PCB (indicated as DMSO control) were 15.1 ± 1.1 and 6.55 ± 0.30 $pmol(hr)^{-1}(mg)^{-1}$ for 2- and 4-$MeOE_2$ production, respectively.

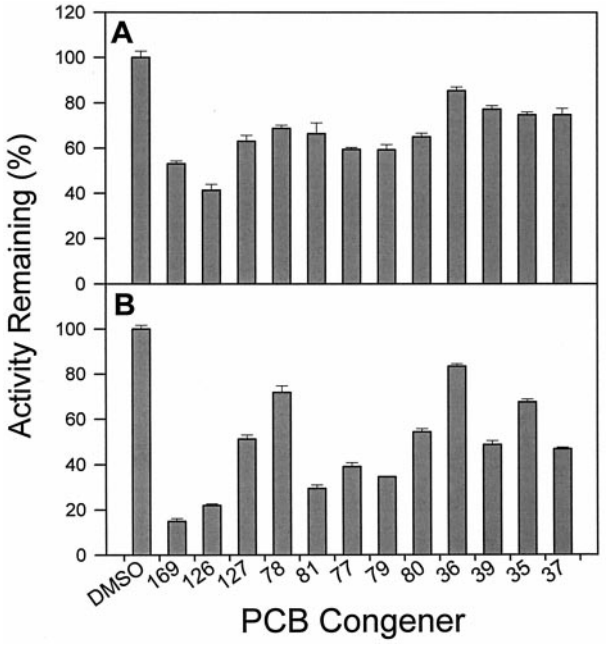

FIG. 7. Inhibition of the TCDD-induced 2- and 4-MeOE$_2$ production by non-*ortho*-substituted PCBs in MCF-7 cells. Cultures were exposed to 10 nM TCDD for 72 hr; then medium was replaced with medium containing 1 μM E$_2$ plus the solvent vehicle (0.1% DMSO) or 1 μM of the indicated PCB congener. Rates of 2-MeOE$_2$ (panel A) and 4-MeOE$_2$ (panel B) formation are expressed relative to the DMSO control; data represent the means ± SEM of three cultures. Rates of TCDD-induced metabolism in the absence of added PCB (indicated as DMSO control) were 20.7 ± 0.5 and 4.50 ± 0.05 pmol(hr)$^{-1}$(mg)$^{-1}$ for 2- and 4-MeOE$_2$ production, respectively.

effectively by PCB 169 than the TCDD-induced 2-MeOE$_2$ production (Fig. 6A). The IC_{50} value for inhibition of 4-MeOE$_2$ was 1.6 nM, whereas the IC_{50} value for inhibition of 2-MeOE$_2$ formation was about 1 μM; the concentration range tested was limited to 1 μM by the low solubility of these PCBs in aqueous media. The TCDD-induced formation of both 2- and 4-MeOE$_2$ was also inhibited by PCB 126, and 4-MeOE$_2$ formation was again inhibited more effectively than 2-MeOE$_2$ formation, although the differences in the IC_{50} values were not as great. PCB 126 inhibited 2- and 4-MeOE$_2$ formation with IC_{50} values of 100 nM and 1 μM, respectively.

Since PCBs 169 and 126 were found to inhibit TCDD-induced E$_2$ metabolism potently, it was of interest to determine whether any other non-*ortho*-substituted PCB congeners had similar effects on E$_2$ metabolism. We found that a number of other congeners had similar inhibitory effects. In Fig. 7, a series of non-*ortho*-substituted PCB congeners were tested at 1 μM for the inhibition of TCDD-induced formation of 2- and 4-MeOE$_2$. All twelve of the congeners tested had inhibitory effects, as each caused at least an 18% inhibition of both 2- and 4-MeOE$_2$ formation in TCDD-treated MCF-7 cells. PCBs 81, 77, 79, 169, and 126 caused at least a 50% inhibition of the TCDD-induced 4-MeOE$_2$ formation, indicating potent inhibition of CYP1B1.

Inhibitory Effects of PCB Congeners on Human CYP1B1

To investigate the possibility that the inhibitory effects of the non-*ortho*-substituted PCB congeners on the TCDD-induced 4-MeOE$_2$ production in MCF-7 cells were due to direct inhibition of CYP1B1, the effects of several of these congeners on the E$_2$ 4-hydroxylase activity of human cDNA-expressed CYP1B1 were determined. The effects of PCBs 169, 126, 78, and 36 at 1 μM are shown in Fig. 8A. Each of the congeners inhibited CYP1B1 activity, and the rank order of inhibition was the same as that observed for the inhibition of the TCDD-induced 4-MeOE$_2$ formation in MCF-7 cells (Fig. 7). The concentration–response relationship for the inhibition of E$_2$ 4-hydroxylation by PCB 169 is shown in Fig. 8B. The IC_{50} value for the inhibition of human cDNA-expressed CYP1B1 by PCB 169 was 11 nM.

DISCUSSION

The goal of this study was to evaluate the potencies of a series of non-*ortho*-substituted PCBs, relative to TCDD, for induction of CYP1A1- and CYP1B1-catalyzed E$_2$ metabo-

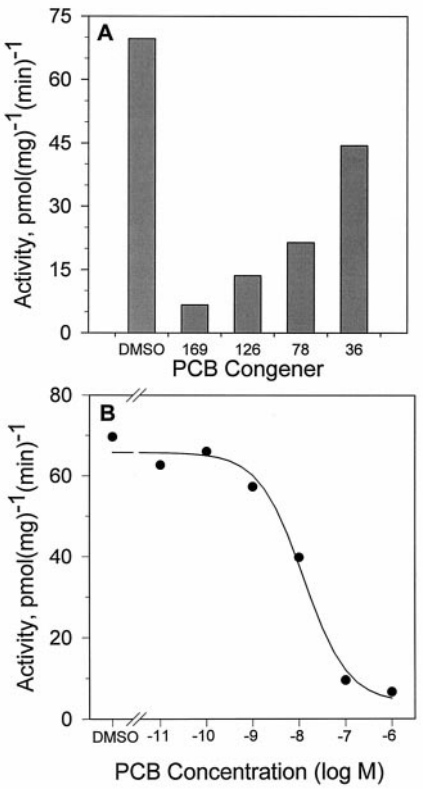

FIG. 8. Inhibition of cDNA-expressed human CYP1B1 by PCBs 169, 126, 78, and 36. Microsomes containing cDNA-expressed human CYP1B1 were preincubated with 5 μM E$_2$ and either the DMSO vehicle, 1 μM of the indicated PCB congener (panel A), or the indicated concentration of PCB 169 (panel B) for 5 min followed by initiation of the reaction by the addition of NADPH. Rates of 4-hydroxyestradiol formation were then determined in a 10-min assay; data represent the means of duplicate determinations.

lism in cultured human cells. In contrast to previous studies with rodents and rodent cells, these results reflect the efficacy with which these compounds activate the human AhR, and thus may provide significant data for estimating TCDD-like toxicity in humans. Our results indicate that the effects of some of the non-*ortho*-substituted PCBs in human cells differ significantly from those previously observed in rodents and rodent cells. These differences in relative potency may reflect differences in the relative binding affinities of the PCB congeners for the human as compared to rodent AhR.

Presently, of the non-*ortho*-substituted congeners, only PCBs 77, 126, and 169 are thought to have sufficient TCDD-like activity, persistence, and abundance in the environment to warrant assignment of TEF values [10, 11]. Our results as well as those of others suggest that PCB 81 may also merit assignment of a TEF value. In the present study, PCB 81 was the most potent inducer of the CYP1 enzymes of the congeners tested. The IEF value for induction of 2-$MeOE_2$ formation relative to TCDD in HepG2 cells was 0.023, which is slightly higher than the value of 0.011 determined for PCB126. The induction of CYP1A1 and CYP1B1 by PCB 81 in HepG2 and MCF-7 cells was evidenced not only by elevated E_2 metabolism, but also by elevated levels of the CYP1A1 and CYP1B1 mRNAs. In recent studies with a wild-type H4IIE rat hepatoma cell line and a recombinant H4IIE cell line containing a luciferase reporter gene under the control of dioxin-responsive enhancers, PCB 81 was found to be potent in eliciting TCDD-like responses [30]. IEF values of 0.0069 for EROD induction in wild-type cells and 0.0027 for stimulation of luciferase activity in the recombinant cells were reported. PCB 81 was less potent than PCB 126, but more potent than PCBs 169 and 77.

PCB 81 also elicits TCDD-like effects in fish and birds. A TEF value of 0.0014 for embryotoxicity in mekada and an IEF value of 0.004 for AHH induction in rainbow trout have been reported; both values are less than that determined for PCB 126 but greater than that determined for PCB 77 [31]. In a number of avian species, PCB 81 has been found to be a potent inducer of embryonic hepatocyte EROD activity, with IEFs of 0.09 to 0.5 [32]. These values are comparable to those determined for PCB 126 (IEFs of 0.06 to 0.3) but somewhat higher than those obtained for PCB 77 (IEFs of $<$0.0003 to 0.07) and PCB 169 (IEFs of 0.002 to 0.02). PCB 81 appears widely dispersed in the environment, as it has been reported to be present in samples of fish [33–37], seal blubber [38], and human breast milk [39] at concentrations lower than those of PCBs 37 and 77 [3], but comparable to those of PCB 126 and usually exceeding those of PCB 169. Taken together, the significant environmental contamination by PCB 81 and the TCDD-like effects of PCB 81 in a number of vertebrate species, including responses in human-derived cells in the present study, support establishment of TEFs for PCB 81 for human and wildlife risk.

In contrast to the effects of PCB 81, our studies indicate minimal effects of PCB 77 on CYP1A1 and CYP1B1 induction in MCF-7 and HepG2 cells. The lack of induction of CYP1A1 by PCB 77 in both cell lines is in agreement with the studies reporting the lack of induction of AHH or EROD activity in the two cell lines by the congener [12]. As noted by Safe [9, 11], responses to PCB 77 are highly variable with regard to individual species and the specific biochemical and toxic effects under investigation. Based on a review of both *in vivo* and *in vitro* studies, Ahlborg and colleagues [10] proposed a TEF value of 0.0005 for PCB 77; a value of 0.01 was suggested by Safe [11]. In the present study, the lack of detectable CYP1A1 or CYP1B1 induction at either the enzyme activity or the mRNA level in HepG2 and MCF-7 cells in response to exposure to 1 μM PCB 77 indicates an IEF value of $<$0.001 in these human cell lines.

The marked induction of CYP1A1 in HepG2 and MCF-7 cells and of CYP1B1 in MCF-7 cells by PCB 39 was unexpected, and has not been reported previously. PCB 39 has not been reported to be a constituent of commercial PCB formulations and has not been detected in environmental samples. Interest in the inductive effects of PCB 39 is focused on evaluation of structure–activity relationships for PCB binding to and activation of the Ah receptor. The proposed model states that chlorine substitution at the two *para*-positions (4 and 4′) of the biphenyl molecule plus two or more *meta*-position (3, 3′, 5, and 5′) substitutions and no *ortho* (2, 2′, 6, and 6′) substitutions are required for maximal TCDD-like activities [11]. The finding that PCBs 39 and 78, other non-*ortho*-substituted congeners with a single *para* substitution, were more potent inducers in MCF-7 cells than PCBs 77 and 169, which have two *para* chlorine substitutions, offers additional structure–activity data to refine the relationships of PCB interactions with the human AhR. Our results showed no inductive effects of a series of *ortho*-substituted congeners when tested at 1 μM; however, some of these congeners might be expected to elicit AhR-mediated effects at higher concentrations. Since the most abundant congeners are *ortho*-substituted [3], toxic equivalency contributions of the lower-potency *ortho*-substituted congeners are also thought to be important [1, 10, 11, 36].

PCB 78 induced both 2- and 4-$MeOE_2$ formation in MCF-7 cells, but failed to induce 2-$MeOE_2$ formation in HepG2 cells. The reason for this difference is unknown. It may reflect subtle differences in the relative binding affinities of AhR proteins expressed in the two cell lines, as both rodent and human AhR are known to be polymorphic [40–42]. A number of human-derived cell lines in addition to MCF-7 and HepG2 that respond to AhR agonists have been identified, including a series of tumor- and non-tumor derived human breast cells that respond to treatment with TCDD by induction of CYP1A1, CYP1B1, or both enzymes [28]. These and other human-derived cell lines could be useful in identifying and characterizing expression of polymorphic forms of the AhR and studying variations in ligand

binding and AhR-mediated responses to PCB congeners that may be due to their expression.

A significant finding of this study was that a number of the non-*ortho*-substituted PCB congeners were inhibitors of human CYP1B1 and, to a lesser extent, human CYP1A1. While inhibitory effects of these congeners on human CYP1B1 and CYP1A1 have not been reported previously, there is evidence for inhibition of CYP1A enzymes of other species by non-*ortho*-substituted PCB congeners. PCB 169 [43] and the brominated analog 3,3′,4,4′,5,5′-hexabromobiphenyl [44] have been shown to be potent inhibitors of rat liver CYP1A2. In a recent study [45], PCBs 77, 126, and 169 were shown to inhibit rat hepatic EROD activity with EC_{50} values in the range of 0.2 to 0.5 μM, and an *ortho*-substituted congener, PCB 153, inhibited the EROD activity with an EC_{50} value only 60-fold higher than those of the non-*ortho*-substituted congeners. PCB 77 and several other congeners also have been shown to inhibit CYP1A enzymes of several species of fish [45–47]. The most potent inhibitory effect observed in this study was that of PCB 169 on CYP1B1. Inhibition of both basal and TCDD-induced 4-$MeOE_2$ production was observed in MCF-7 cells, and the effect on CYP1B1 was confirmed in experiments with the cDNA-expressed enzyme. It was also observed that CYP1B1 was much more sensitive to inhibition by PCB 169 than CYP1A1, as the EC_{50} value for inhibition of 4-$MeOE_2$ formation was approximately 600-fold lower than for inhibition of 2-$MeOE_2$ formation (Fig. 6A). This selective inhibition of CYP1B1 may be useful in some cases in distinguishing between CYP1B1- and CYP1A1-catalyzed metabolism.

The inhibitory effects of the non-*ortho*-substituted PCBs also have implications for determinations of relative potency and evaluations of additive and antagonistic effects of PCBs that are based on activity measurements. Since CYP1A1-catalyzed activities, AHH and EROD, are the most commonly used parameters to evaluate relative potencies of PCBs [11], enzyme inhibition may have significant effects in these determinations. In some cases, the observed enzyme activities may reflect the net result of inductive and inhibitory effects, and may explain in part non-additive effects of PCBs on enzyme induction. Direct inhibition of the induced enzymes would be another mechanism to explain antagonistic effects of PCBs on induction of CYP1A1-catalyzed activities, in addition to effects on binding to and activation of the AhR [12]. In this study, PCBs 37 and 77 failed to induce human CYP1A1 or CYP1B1 at 1 μM, but both congeners inhibited human CYP1A1- and CYP1B1-catalyzed E_2 metabolism at this concentration. Since PCBs 37 and 77 are two of the more abundant non-*ortho*-substituted congeners in the environment [3], these congeners may actually contribute to reduced human CYP1 activities in the relative potency determinations of mixtures.

The potent inhibitory effects of the non-*ortho*-substituted congeners on CYP1B1 and CYP1A1 activities suggest high-affinity binding of these congeners to the active sites of CYP1B1 and CYP1A1, and raise the possibility that some of the congeners may be substrates for these enzymes. Hydroxy-PCB metabolites often have biological effects that are distinct from those of the parent PCB. Some hydroxy-PCB metabolites bind the estrogen receptor [48, 49], elicit estrogenic effects *in vitro* [49–51], and may mediate the uterotropic effects of PCBs observed *in vivo* in rats [52, 53]. The conversion of PCB 77 to an estrogenic metabolite may account for the estrogenicity of the congener that has been observed *in vivo* in mice and in MCF-7 cells [54]. In addition to estrogenic effects, some hydroxy-PCBs also appear to interfere with thyroid hormone function [55].

High-affinity binding of poorly metabolized compounds including TCDD and some of the highly chlorinated PCB congeners may cause additional biological and toxic effects. Both TCDD [56] and PCB 169 [43] are known to bind tightly to rat hepatic CYP1A2. The major protein binding site for sequestration of TCDD in rat liver was shown to be CYP1A2 [56]. The failure of these compounds to accept active oxygen may lead to futile catalytic cycles [57] that can be a source of reactive oxygen species and free radicals, ultimately leading to damage to cellular macromolecules. The high-affinity interaction between the poorly metabolized PCB 169 and human CYP1B1 reported here may cause similar futile catalytic cycles in the tissues in which CYP1B1 is expressed, including kidney, uterus, mammary gland, and prostate [26, 58].

In summary, non-*ortho*-substituted PCBs not only are inducers of P450s of human CYP1A1 and CYP1B1, but also inhibit their activities as well. It appears that enzyme induction, enzyme inhibition, and the possible conversion of PCBs to metabolites with estrogenic or other physiologic activities must be considered in evaluating the biochemical, endocrine, and toxic effects of these chemicals.

This work was supported by Grants ES04913 (Superfund Basic Research), CA70553, ES08148, and Center Grant ES03819. C. L. Hayes was supported by Training Grant ES07141. The excellent technical assistance of Ying Li is gratefully acknowledged.

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