Hydroxylated Polychlorinated Biphenyls Identified in Human Serum as Inducers and Inhibitors of EROD Activity in the H4IIE Cell Bioassay

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Abstract

Eight hydroxylated PCBs that have been identified in human serum were tested in the H4IIE bioassay for induction of ethoxyresorufin-O-deethylase activity (EROD). The compounds were also cotreated with 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) to determine their effects on TCDD induced EROD activities. The eight hydroxy-PCBs tested were respectively: 2',3',4',5'-tetrachloro-4-biphenylol, 2,3,3',4',5-pentachloro-4-biphenylol, 2,2',3,4',5,5' hexachloro-4-biphenylol, 2,2',3',4,4',5,5'-heptachloro-3-biphenylol, 2',3,3',4',5-pentachloro-4-biphenylol, 2,2',3,3',4',5-hexachloro-4-biphenylol, 2,2',3,3',4',5,5'-heptachloro-4-biphenylol, and 2,2',3,4',5,5',6-heptachloro-4-biphenylol. Hydroxy-PCBs 1 and 5 at $2x10^{-6}$ M concentrations induced > 60 percent of the EROD activity observed after treatment with $2x10^{-9}$ M TCDD. Hydroxy-PCB 4 was inactive as an inducer of EROD activity, while hydroxy-PCBs 3 and 7 caused only a 26 and 13% induction relative to the maximal TCDD-induced response, respectively. When cells were cotreated with the hydroxy-PCBs and $2x10^{-9}$ TCDD, none of the OH-PCBs significantly decreased the TCDD-induced EROD activities. The results of this study indicate that the hydroxy-PCBs can be partial agonists for induction of EROD activity, but they do not antagonize TCDD induced activities.

Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants and have been quantitated in both biotic and abiotic compartments including sediments, air, fish, wildlife, and human adipose tissue, breast milk and serum [1,2]. The biochemical and toxic effects of PCBs have been extensively studied and have been previously reviewed[3]. While up to 132 compounds have been identified in PCBs mixtures, the nonortho or coplanar PCBs display toxic and biochemical responses which segregate with the aryl hydrocarbon (Ah) receptor and thus are similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The H4IIE rat hepatoma cell bioassy where CYP1A1-mediated induction of EROD activity is quantitated has been used to characterize the induction potency of various halogenated aromatic hydrocarbons such as PCBs, polychlorinated dibenzodioxins and furans [4-7]. H4IIE cells have low basal CYP1A1-dependent activity but are highly inducible by HAHs. Because the various HAH congeners bind to the Ah receptor and elicit a number of common toxic and biochemical responses, a toxic equivalency (TEQ) approach has been developed to describe the potency of complex mixtures of HAHs. 2,3,7,8-TCDD is the most potent inducer of EROD activity

in H4IIE cells and is used as a reference standard for developing toxic equivalency factors (TEFs) for the induction activity of individual HAHs [8,9].

In this study the H4IIE bioassay was used to assess the EROD induction potential of a series of hydroxylated PCBs which have been identified as contaminants in human serum. It has been reported that the hydroxy-PCBs of coplanar PCBs exhibit significantly lower Ah-receptor agonist activities than their parent hydrocarbon and exhibit lower toxicities [10,11]. Hydroxylated PCBs have been reported to inhibit mitochondrial oxidative phosphorylation in rodents, to bind with high affinity to transthyretin a serum thyroxine transport protein[12], and some congeners bound to the estrogen receptor and elicit estrogenic responses in the female rat uterus while others have antiestrogenic activities[13,14]. Six of the hydroxy-PCBs showed partial induction of EROD activity (compared to TCDD), however none of the hydroxy-PCBs congeners antagonized TCDD induced EROD activity in rat hepatoma H4IIE cells.

Experimental Methods

Chemicals

The eight OH-PCBs used in this study were synthesized in this laboratory as previously described [15]. The compounds (Figure 1) were numbered 1-8 for convenience and are as follows: 2',3',4',5'-tetrachloro-4-biphenylol, 2,3,3',4',5-pentachloro-4-biphenylol, 2,2',3,4',5,5' hexachloro-4-biphenylol, 2,2',3',4,4',5,5'-heptachloro-3-biphenylol, 2',3,3',4',5-pentachloro-4-biphenylol, 2,2',3,3',4',5-hexachloro-4-biphenylol, 2,2',3,3',4',5,5'-heptachloro-4-biphenylol, and 2,2',3,4',5,5',6-heptachloro-4-biphenylol. The compounds were >97% pure as determined by gas-chromatographic analysis. Compounds were dissolved into DMSO for treating the cells.

Cell Culture and EROD Assay

Rat hepatoma H4IIE cells were grown as a continuous cell line in a-minimum essential media (Sigma, St. Louis, MO) supplemented with 2.2 g/L sodium bicarbonate, 10% fetal bovine serum, and 10 ml/L antibiotic/antimycotic solution. Stock culture cells were grown in 150 cm^2 plates at 37°C in a humidified air/carbon dioxide (95/5%) atmosphere. Cells were seeded into 48 well plates at a density of 80,000 cells per well in 0.5 ml media. After 24 hr, plates were treated with 1 µl per well of the OH-PCBs 1-8 (shown in Figure 1) plus either 1 µl of DMSO or 1 µl TCDD. Treatments were as follows: $2x10^{-6}$ OH-PCB, $2x10^{-7}$ OH-PCB, $2x10^{-8}$ OH-PCB, $2x10^{-6}$ OH-PCB + $2x10^{-9}$ TCDD, $2x10^{-7}$ OH-PCB + $2x10^{-9}$ TCDD, $2x10^{-8}$ OH-PCB + $2x10^{-9}$ TCDD, $2x10^{-9}$ OH-PCB + $2x10^{-9}$ TCDD, $2x10^{-9}$ TCDD, and DMSO. Treatments were run in triplicate on a plate and at least three plates per treatment were analyzed. EROD activity and protein concentrations were determined as described[16] on a CytoFluor 2350 plate reader. Plates were read at 530 nm/590 nm for resorufin production and 400 nm/460 nm for fluorescamine protein determination.

Results and Discussion

The relative EROD induction of the eight hydroxy-PCBs ($2x10^{-6}$ M) compared to TCDD treatment ($2x10^{-9}$ M) is shown in Figure 2. Hydroxy-PCB 5 was the most potent inducer of EROD activity with 73% of the response observed for $2x10^{-9}$ M TCDD. Hydroxy-PCBs 6 and 1 induced 53 and 61% of this response. In contrast, hydroxy-PCB 4 was inactive as an inducer and hydroxy-PCB 7 induced minimal activity (13%). Hydroxy-PCB 4 is the only PCB tested which contains a

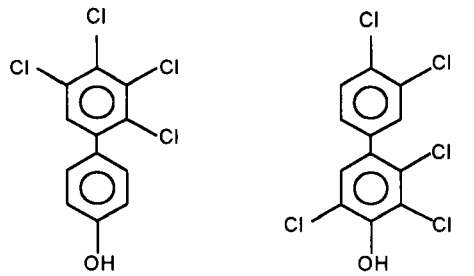
-3-biphenylol and this may play a role in decreased Ah receptor binding affinity of this congener. Hydroxy-PCB 1 competitively binds the estrogen receptor [13,14], and in this study it was one of the more potent inducers of EROD activity.

Previous studies have indicated that some PCBs such as PCB 77 (3,3',4,4'-tetrachlorobiphenyl) can inhibit EROD activities. In PLHC-1 cells, EROD activity was reduced with 1 and 10 μM PCB 77 compared to the 0.1 μM treatment [17]. In our study there was a dose-dependent increase in EROD activity between $2x10^{-8}$ and $2x10^{-6}$ M treatments for all active hydroxy-PCBs. Furthermore, in cotreatment studies $2x10^{-6}$ M concentrations of hydroxy-PCB plus $2x10^{-9}$ M TCDD did not significantly decrease EROD induction compared to $2x10^{-9}$ TCDD treatment alone (Figure 3).

In conclusion, certain hydroxy-PCBs were significant inducers of EROD activity in H4IIE rat hepatoma cells. Therefore, these compounds could influence TEQ bioassay results of samples containing mixtures of hydroxy-PCBs and other HAHs. However, at 1000-fold higher concentrations the hydroxy-PCBs do not appear to inhibit TCDD induced EROD activities.

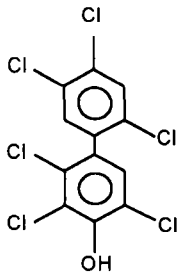
Acknowledgment

The financial assistance of the National Institutes of Health (ES04917) and the Texas Agricultural Experiment Station are gratefully acknowledged.



OH-PCB 1

OH-PCB 2



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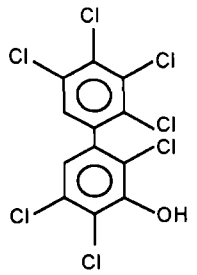
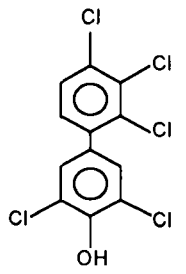
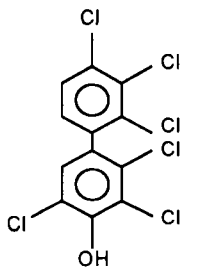


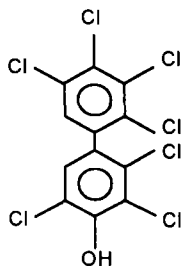
OH-PCB 4



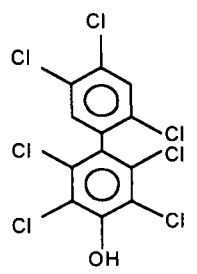
OH-PCB 5



OH-PCB 6



OH-PCB 7



OH-PCB 8

Figure 1. Structures of the hydroxylated PCBs identified in human serum and used in this study.

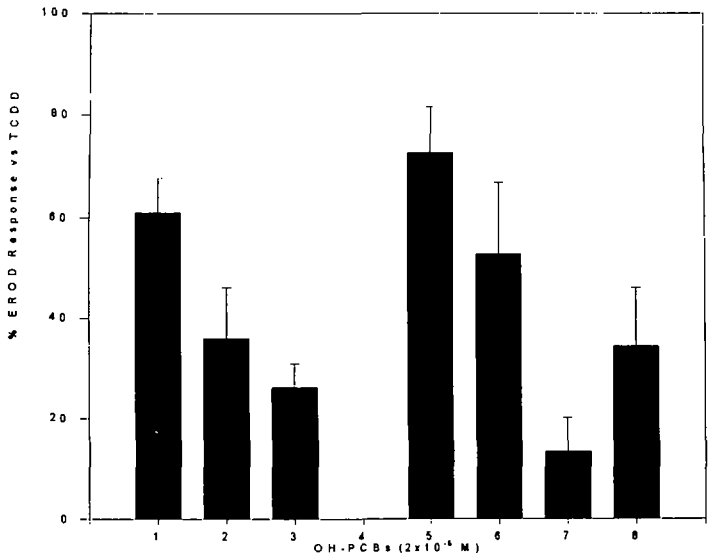


Figure 2. EROD induction potency of the 8 OH-PCBs ($2x10^{-6}$ M) expressed relative to $2x10^{-9}$ M TCDD. EROD activities and protein determinations were determined in triplicate on a 48-well plate using a CytoFluor 2350 plate reader as described in the Materials and Methods section.

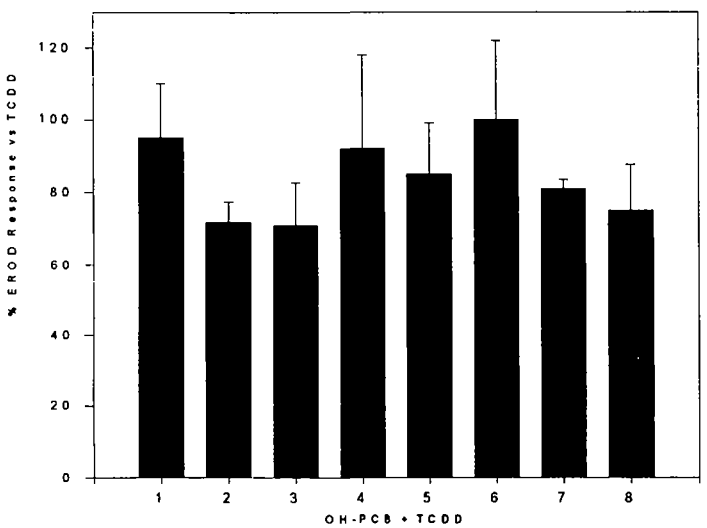


Figure 3. Interaction of OH-PCBs and TCDD on EROD activities. Cotreatments of $2x10^{-6}$ M OH-PCB + $2x10^{-9}$ M TCDD for each of the 9 OH-PCBs are shown relative to EROD activities for $2x10^{-9}$ TCDD alone (= 100%).

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