In Vitro Bioassays for Polychlorinated Diphenyl Ethers: Quantitative Structure-Activity Relationships

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ABSTRACT

Treatment of rat hepatoma H-4-II E cells with six polychlorinated diphenyl ether (PCDE) congeners resulted in a concentration-dependent induction of ethoxyreorufin *O*-deethylase (EROD) activity, an indicator of CYPIA1 gene expression. EC_{50} values obtained from the induction curves revealed the following order of induction potencies: 2,4,5-3',4'-pentaCDE (9 nM), 3,4-3',4'-tetraCDE (20 nM), 3,4,5-3'4'-pentaCDE (70 nM), 2,3,4,5-3',4'-hexaCDE (70 nM), 2,4,5-2',4',5'-hexaCDE (330 nM), and 2,4-2',4',5'-pentaCDE (>10 μM). Results from gel shift anaysis indicated that these compounds are capable of activating the aryl hydrocarbon recepor (AhR) from rat hepatic cytosol to a DNA-binding form. In addition, these PCDE congeners inmhibited 17β-estradiol (E_2)-induced secretion of the 52 kDa protein in MCF-7 human breast cancer cells. Moreover, the same general structure-activity relationship observed for induction of EROD activity was also seen for for PCDE-mediated antiestrogenicity. These results suggest that the AhR-mediated biological effects of lower chlorinated diphenyl ethers can be measured in vitro.

INTRODUCTION

PCDEs are ubiquitous environmental contaminants which are formed as by products in the synthesis of chlorophenols. Chlorophenols are used widely as wood preservatives, fungicides, and intermediates in the synthesis of phenoxyacetic acid herbicides[1]. A variety of PCDE congeners have been identified in fish, wildlife, and human tissues[1,2]. Like polychlorinated biphenyls (PCBs), PCDEs are hydrophobic in nature and relatively stable and therefore have high potential for bioaccumulation. Because little research has focused on the toxicityof PCDEs, the environmental impact and toxicological significance of the release of these compounds into the environment is unknown.

MATERIALS AND METHODS

Treatment of cells. Rat hepatoma H-4-II E cells were maintained in α-MEM medium supplemented with 2.2 mg/ml tissue culture grade $NaHCO_3$, 5% fetal calf serum, and 10 ml/l antibiotic/antimycotic solution. MCF-7 human breast cancer cells were maintained in MEM medium supplemented with 2.2 mg/ml tissue culture grade $NaHCO_3$, 6 μg/ml insulin, 100 μl Na pyruvate (1 mM), 2.35 g/l HEPES buffer, 5% fetal calf serum, and 10 ml/l antibiotic/antimycotic solution. Stock cultures were grown in 150-cm^2 tissue culture flasks and incubated in a humidified mixture of 5% CO_2 and 95% air under atmospheric pressure.

52 kDa protein assay. Detection and analysis of the 52 kda protein was performed as previously described[3].

Ethoxyresorufin O-deethylase (EROD) assay. Cells were harvested by manual scraping from the plate and the EROD activities determined by the methods of Pohl and Fouts[4].

Gel shift assay. Rat hepatic cytosol (1 mg/ml protein) was treated with PCDEs such that the final concentration of DMSO was 1% (v/v). Gel shift anaysis was performed as previously described[5].

RESULTS

Rat hepatoma H-4-II E cells were treated with a series of six PCDEs (10^{-6}-10^{-9} M). A concentration-dependent increase in EROD activity was observed for all congeners except 2,4,5-2',5'-pentaCDE (figure 1). EC_{50} values correlated well with in vivo induction potencies reported by Howie et al[6] (table 1). Gel shift analysis of rat hepatic cytosol treated with PCDEs (10^{-5}M) indicated that these congeners are capable of transforming the AhR to a DNA binding form (figure 4, table 1). In addition, treatment of MCF-7 human breast cancer cells with these PCDE compounds resulted in a concentration-dependent decrease in E_2-stimulated secretion of a 52 kDa protein (figure 2). Moreover, the antiestrogenic effects of these PCDE congeners correlated well with their capacity to induce EROD activity (figure 3).

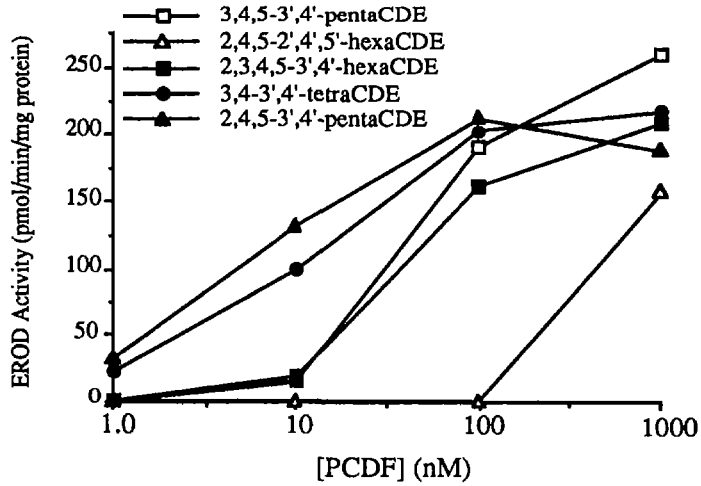

Figure 1. Concentration-dependent induction of EROD activity by PCDE congeners in H-4-II E cells

Treatment	EROD Induction (EC_{50})	Relative DRE Binding	AntiE_2(EC_{50})
2,4,5-3'4'-pentaCDE	9 nM	73.2	80 nM
3,4-3',4'-tetraCDE	20 nM	72.0	1000 nM
3,4,5-3',4'-pentCDE	70 nM	34.4	950 nM
2,3,4,5-3',4'-hexaCDE	70 nM	49.9	1000 nM
2,4,5-2',4',5'-hexaCDE	330 nM	11.7	1050 nM
2,4,5-2',5'-pentaCDE	>10,000 nM	7.3	> 10,000 nM

Table 1. Potency of PCDE congeners to transform the AhR to a DNA-binding form, induce EROD activity, and inhibit secretion of the 52 kDa protein.

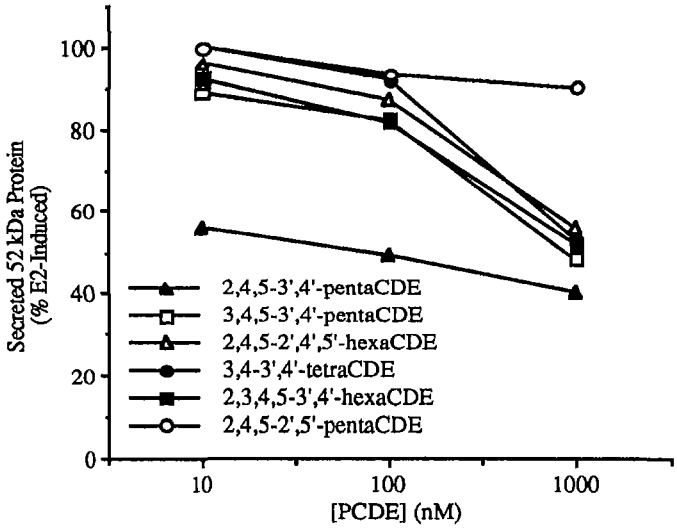

Figure 2. Reduction of E_2-stimulated secretion of the 52 kDa protein by PCDE congeners in MCF-7 cells.

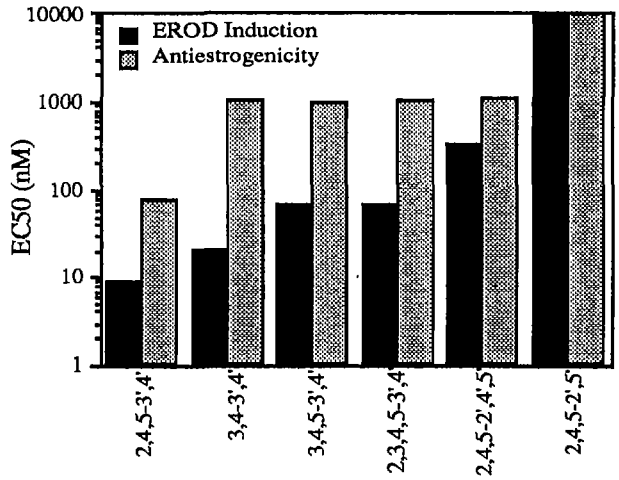

Figure-3. Correlation of the potency of six PCDF congeners to induce EROD activity in H-4-II E cells and inhibit E_2-stimulated secretion of the 52 kDa protein in MCF-7 cells.

CONCLUSIONS

Iverson and coworkers have proposed that PCDEs elicit toxic and biological responses (including EROD induction) at the cellular level by binding to the AhR[7]. Induction of P450IA1 enzyme activitites by PCDEs is a result of the coplanarity of the phenyl rings in congeners which have no *ortho* substituents. As reported for PCBs, *ortho*-substitution significantly hinders the formation of coplanar conformers. The *ortho*-effect is not as important for PCDEs as for PCBs, and this is due to the ether linkage providing more space for rotation of the rings[7]. Like the PCBs, the potency of PCDE congeners to induce EROD

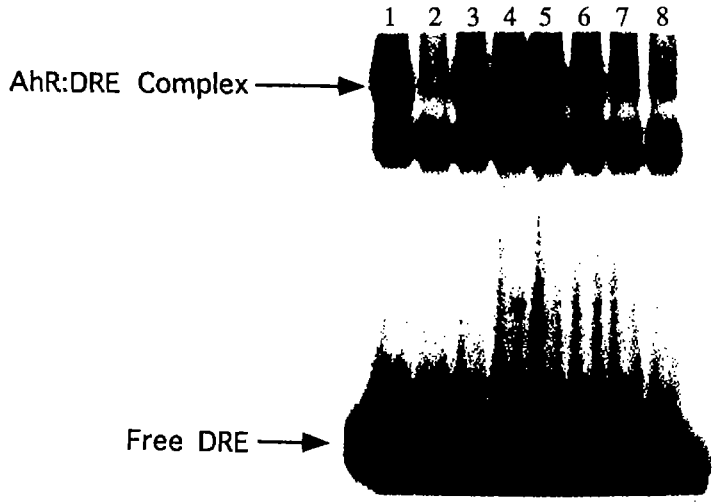

Figure 4. Gel shift analysis of PCDE:AhR complexes binding to the DRE. Lane 1) TCDD 2) DMSO 3) 2,4,5-3',4'-pentaCDE 4) 3,4-3',4'-tetraCDE 5) 3,4,5-3'4'-pentaCDE 6) 2,3,4,5-3',4'-hexaCDE 7) 2,4,5-2',4',5'-hexaCDE 8) 2,4-2',4',5'-pentaCDE

activity is also enhanced for compounds which contain chlorines at both para and at least one meta position. The data presented in this study are consistent with this theory. Also, results from structure-binding and structure-induction support an AhR-mediated induction of EROD activity by PCDE compounds.

The potency of these PCDE congeners to induce EROD activity in cultured H-4-II E cells and their antiestrogenic activity in MCF-7 human breast cancer cells correlated well their activities as inducers of EROD activity in C57BL/6 mice[6]. In addition, the potency of these compounds to induce both biological effects correlated well with their abilities to transform the AhR to a DNA-binding form (table 1). These results indicate that the biological and toxic effects of the lower chlorinated PCDEs can be measured using at least two different in vitro cell culture assays.

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