Relative Contributions of Affinity and Intrinsic Efficacy to Aryl Hydrocarbon Receptor Ligand Potency

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The aryl hydrocarbon receptor (AHR) is a soluble receptor/transcription factor that mediates the toxicity of a variety of compounds, most notably 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and structurally related halogenated aromatic hydrocarbons (HAH). Individual HAHs differ dramatically in their potency for eliciting biological effects. Application of receptor models based on pharmacological principles to evaluate relationships among chemical structures and biological potencies of AHR ligands may aid in predicting their toxicity, both individually and in mixtures (Poland, 1991, 1996). A "Receptor Biology Roundtable" has called for quantitative assessment of ligand-receptor interactions to aid in mechanism-based risk assessment of environmental toxicants (Limbird and Taylor, 1998).

The potency of the ligand for eliciting a response (i.e., the dose-response relationship) depends on the properties of affinity and efficacy. Affinity is the strength of the interaction, or binding, with the receptor, and is a property of the ligand and receptor. Efficacy is the ability of that ligand-receptor complex to produce a response (Ariens, 1954; Stephenson, 1956) and is influenced by both ligand- and tissue-specific properties. The intrinsic efficacy of a ligand is the ability of that ligand to convert the receptor to an active form (Furchgott,


Models of receptor action are valuable for describing properties of ligand-receptor interactions and thereby contribute to mechanism-based risk assessment of receptor-mediated toxic effects. In order to build such a model for the aryl hydrocarbon receptor (AHR), binding affinities and CYP1A induction potencies were measured in PLHC-1 cells and were used to determine intrinsic efficacies for 10 halogenated aromatic hydrocarbons (HAH): 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), and eight polychlorinated biphenyls (PCB). TCDD, TCDF, and non-ortho-substituted PCBs 77, 81, 126, and 169 behaved as full agonists and displayed high-intrinsic efficacy. In contrast, the mono- and di-ortho-substituted PCBs bound to the AHR but displayed lower or no intrinsic efficacy. PCB 156 was a full agonist, but with an intrinsic efficacy 10- to 50-fold lower than non-ortho-substituted PCBs. PCB 118 was a very weak partial agonist. PCBs 105 and 128 were shown to be competitive antagonists in this system. The model was then used to predict CYP1A induction by binary mixtures. These predictions were tested with binary mixtures of PCB 126, 128, or 156 with TCDD. Both PCB 156 (a low-intrinsic efficacy agonist) and PCB 128 (a competitive antagonist) inhibited the response to TCDD, while the response to TCDD and PCB126 was additive. These data support the following conclusions: 1) only 1-2% of the receptors in the cell need be occupied to achieve 50% of maximal CYP1A induction by one of the high-intrinsic efficacy agonists, demonstrating the existence of "spare" receptors in this system; 2) the insensitivity of fish to ortho-substituted PCBs is due to both reduced affinity and reduced intrinsic efficacy compared to non-ortho-substituted PCBs; 3) PCB congeners exhibit distinct structure-affinity and structure-efficacy relationships. Separation of AHR ligand action into the properties of affinity and intrinsic efficacy allows for improved prediction of the behavior of complex mixtures of ligands, as well as mechanistic comparisons across species and toxic endpoints.

3 Abbreviations used: AHR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; BSA, bovine serum albumin; CYP1A1, cytochrome P4501A1; DRE, dioxin responsive element; EROD, ethoxyresorufin-O-deethylase; HAH, halogenated aromatic hydrocarbon; LSC, liquid scintillation counting; MEM, minimum essential medium; PBS, phosphate-buffered saline; PCB, polychlorinated biphenyl; PCB 77, 3,3',4,4'-tetrachlorobiphenyl; PCB 81, 3,4,4',5-tetrachlorobiphenyl; PCB 105, 2,3,3',4,4'-pentachlorobiphenyl; PCB 118, 2,3',4,4',5-pentachlorobiphenyl; PCB 126, 3,3',4,4',5-pentachlorobiphenyl; PCB 128, 2,2',3,3',4,4'-hexachlorobiphenyl; PCB 153, 2,2',4,4',5,5 '-hexachlorobiphenyl; PCB 156, 2,3,3',4,4',5-hexachlorobiphenyl; PCB 169, 3,3',4,4',5,5'-hexachlorobiphenyl; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran; TEF, toxic equivalency factor.

4 We have followed the definitions of these and other terms related to receptor pharmacology as outlined in Jenkinson et al. (1995).
AFFINITY AND EFFICACY OF AHR LIGANDS

Thus, individual ligands can be characterized by their affinity for the receptor and by the intrinsic efficacy with which they activate the receptor. Tissue-specific properties (often collectively termed “coupling”) include the concentrations of receptors and other molecules required for transduction of the signal initiated by the ligand–receptor complex to achieve a response.

The best-studied and most frequently used measure of response to AHR agonists is cytochrome P4501A (CYP1A) induction. Following agonist binding, the AHR translocates to the nucleus, forms a heterodimer with ARNT, and interacts with enhancer elements (DREs) and transcriptional cofactors to activate transcription of several genes, including CYP1A1 (for reviews see Hankinson, 1995; Schmidt and Bradfield, 1996).

Based largely on CYP1A induction, the relationships between chemical structure and response potencies for several AHR ligands have been evaluated (for review see Safe, 1990). These studies have resulted in identification of agonists, partial agonists (Blank et al., 1987; Astroff et al., 1988; Merchant et al., 1992; Santostefano et al., 1992), and antagonists (Biegel et al., 1989; Aarts et al., 1995; Lu et al., 1995; Gasiewicz et al., 1996; Reiners et al., 1998; Ciolino et al., 1999; Henry et al., 1999). AHR binding affinities, response EC50s, and/or response inhibition IC50s have been determined for some of the compounds, but intrinsic efficacies of AHR ligands have not. A quantitative assessment of intrinsic efficacies is necessary to construct mechanistic models of AHR ligand action.

Here we report the characterization of AHR ligands in a system where stimulus (AHR binding) and response (CYP1A induction) were measured in whole-cell assays. The data were used to build a stimulus–response model for the cells of interest. The primary use of the model was to develop a general pharmacological approach to distinguish the contributions of affinity and intrinsic efficacy to AHR ligand potency. The utility of this approach then was demonstrated by using these data to determine the molecular basis for the relative insensitivity of fish to ortho-substituted polychlorinated biphenyls (PCBs). Reduced potency of these compounds in fish was first suggested by in vivo studies of CYP1A inducibility (Gooch et al., 1989) and later supported by studies ofembryotoxicity (Walker and Peterson, 1991) and CYP1A induction in vitro (Hahn and Chandran, 1996).

The PLHC-1 cell line, derived from a hepatocellular carcinoma of the teleost Poeciliopsis lucida (Hightower and Renfro, 1988), expresses an AHR and an inducible CYP1A1 (Hahn et al., 1993, 1996; Hestermann et al., 2000). Moreover, PLHC-1 cells, like fish and other fish cells, are relatively insensitive to ortho-substituted PCBs, as measured by CYP1A induction and uroporphyrin accumulation (Hahn and Chandran, 1996; Hahn and Woodward, unpublished results). Therefore, these cells are an appropriate system for testing the mechanism of insensitivity. Ten HAH, including TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and non-ortho-, mono-ortho-, and di-ortho-substituted PCBs, were chosen to include known agonists as well as suspected partial agonists and antagonists. Furthermore, these compounds span a range of toxic potencies and many of them occur in the environment at concentrations sufficient to warrant concern for their effects in wildlife and humans (Jones, 1988; McFarland and Clarke, 1989; van den Berg et al., 1998).

We show here that the insensitivity of PLHC-1 cells to ortho-substituted PCBs is due to differences in both affinity and intrinsic efficacy compared to non-ortho-substituted PCBs. Stimulus–response relationships determined for these HAH broaden our understanding of the activities of these compounds and provide a framework for future studies of other organisms, tissues, AHR ligands, and responses.

MATERIALS AND METHODS

Chemicals and solutions. 2,3,7,8-Tetrachlorodibenzo-p-dioxin ([3H]TCDD, purity > 97%, specific activity 27 Ci/mmol) was obtained from Chemsyn Science Laboratories (Lenexa, KS). TCDD, TCDF, and all PCBs (purity > 98% for all) were obtained from Ultra Scientific (Kingston, RI). Resorufin, ethoxyresorufin, and Amplex Red were from Molecular Probes (Eugene, OR). Peroxidase conjugated goat anti-mouse antibody was from Pierce (Rockford, IL). All other reagents were obtained from Sigma (St. Louis, MO).

Phosphate-buffered saline (PBS) is 0.8% NaCl, 0.115% Na2HPO4, 0.02% KCl, 0.02% KH2PO4, pH 7.4. Phosphate buffer is 50 mM NaHPO4, with pH adjusted to 8.0 using 50 mM Na2HPO4. TCDD, TCDF, and PCB solutions were prepared in dimethyl sulfoxide as described previously (Hahn et al., 1996). Concentrations of [3H]TCDD solutions were verified by liquid scintillation counting (LSC) on a Beckman LS5000TD.

Growth and treatment of cells. PLHC-1 cells (Hightower and Renfro, 1988) were grown at 30°C in minimum essential medium (MEM) containing Earle’s salts, nonessential amino acids, 1-glutamine, and 10% calf serum, as described previously (Hahn et al., 1993). These cells express a single CYP1A isoform, which has no detectable constitutive expression (Hahn et al., 1996). These cells also express only one AHR, an AHR2 form (Hahn, 1998; Hestermann et al., 1998). For EROD and CYP1A ELISA assays, cells were seeded into 96-well plates (Costar, Cambridge, MA) at 2 x 10^5 cells in 0.2 mL culture medium per well. One day later the medium was removed and replaced with 0.2 mL serum-free MEM. The cells were then treated by addition of solutions dissolved in DMSO or DMSO alone (1 μL/well). DMSO concentrations were ≤0.5% (v/v) in all treatments and did not affect cell viability. Following treatment, plates were incubated at 30°C for 24 h. For TCDD-specific binding experiments, [3H]TCDD and competitors were dissolved at twice the desired concentration in 0.75 mL serum-free MEM in glass tubes. Cells were trypsinized and resuspended at 2 x 10^6 cells/mL in serum-free MEM, and 0.75 mL cell suspension was added to each tube. Aliquots of cell suspension were reserved for protein determination.

EROD and protein assays. EROD activity was measured using a multiwell fluorescence plate reader by a modification of the method of Kennedy et al. (1995). Cells were rinsed once with 0.2 mL room temperature PBS, and the
EROD reaction was then initiated with the addition of 2 \( \mu \text{M} \) 7-ethoxyresorufin in phosphate buffer (100 \( \mu \text{M} \) well). The reaction was stopped after 8 min (resorufin production is linear with respect to time over this period; Hahn et al., 1996) with the addition of 75 \( \mu \text{l} \) ice-cold fluorescein solution (0.15 mg/ml in acetonitrile). After a 15-min incubation, resorufin and fluorescein fluorescence were measured. Resorufin and protein concentrations were determined from standard curves prepared on the same plate. BSA was used for the protein standard.

For the TCDD binding experiments, cell protein was measured by the bicinechonic acid method of Smith et al. (1985), using BSA as the standard and MEM as the blank.

**ELISA assay.** Enzyme-linked immunosorbent assays to detect CYP1A were performed essentially as described by Brischweiler et al. (1996). One day after treatment in 96-well plates, cells were fixed in 50% ethanol for 15 min, in 75% ethanol for 15 min, and in 95% ethanol for 30 min. After washing with PBS, nonspecific antibody binding was blocked with 10% fetal bovine serum and 2% BSA in PBS for 1 h. The primary antibody, mouse anti-scup CYP1A monoclonal antibody 1-12-3 (10 \( \mu \text{g/ml} \); Park et al., 1986), was then added in 100 \( \mu \text{l} \) blocking solution for 1 h. After three washing steps with PBS, 100 \( \mu \text{l} \) secondary antibody, peroxidase conjugated goat anti-mouse (1:1000 in blocking solution), was added for 1 h. After another three washing steps with PBS, 100 \( \mu \text{l} \) substrate solution (100 \( \mu \text{M} \) Amplex Red, 100 \( \mu \text{M} \) H2O2 in phosphate buffer, pH 7.0) was added for 30 min. All incubations were performed at room temperature.

Resorufin formation was measured in the fluorescence plate reader. For each treatment, the background fluorescence, defined as the fluorescence detected in untreated cells, was subtracted, and all values were normalized to the maximum response measured. The assay was also performed on wells without cells or without the addition of primary antibody, and these controls yielded fluorescence values nearly identical to those in untreated cells, consistent with our earlier results detecting no CYP1A protein or EROD activity in untreated cells (Hahn et al., 1996).

**TCDD and competitor binding to the AHR.** Specific binding of \( ^{[1]}\text{H} \) TCDD in PLHC-1 cells was measured by a modification of the whole-cell filtration assay of Dold and Greenlee (1990). For determination of the equilibrium dissociation constant \( (K_d) \) of TCDD binding to the AHR and the receptor content \( (R_c) \) of PLHC-1 cells, the cells were treated with increasing concentrations of \( ^{[1]}\text{H} \) TCDD in the presence or absence of 200-fold molar excess of unlabeled TCDF and incubated for 2 h at 30°C. This time was determined to be sufficient to achieve a steady state of bound radioligand (Hestermann, 1999). For determination of binding inhibition constants \( (K_i) \), 0.5 to 1 nM \( ^{[1]}\text{H} \) TCDD and increasing concentrations of competitors (or a 200-fold excess TCDF treatment to measure nonspecific binding) were dissolved in MEM. Cells suspended in MEM were subsequently added to ensure true competition, since off rates for AHR ligands can be extremely slow (Farrell and Safe, 1987). Cell densities were equal among experiments in order to minimize protein concentration effects on binding (Bradfield et al., 1988). Following the incubation, tubes were vortexed briefly to assure even distribution of cells, and a 0.1-ml aliquot was removed to determine final concentration of \( ^{[1]}\text{H} \) TCDD concentration. Three 0.45-ml aliquots of cell suspension from each tube were then collected under vacuum on prewetted 25-mm Whatman GF/F filters. In some cases cell aliquots were pelleted (200g, 10 min) and resuspended in PBS prior to application to filters. Filters were then washed three times with 2.5 ml acetone that had been precooled to ~80°C. The number of washes was determined empirically as that necessary to remove the free \( ^{[1]}\text{H} \) TCDD remaining on the filters. Replicates were processed in batches of 12 on a Millipore 1225 filter manifold. Radioactivity remaining on the filter was quantified by LSC.

**Data analysis and theoretical models.** EROD data were fit to a modified Gaussian function for determination of dose–response relationships, as described previously (Kennedy et al., 1993; Hahn et al., 1996). CYP1A induction data were fit to the Hill response function

\[
\frac{[\text{CYP1A}]}{[\text{CYP1A}_{\text{max}}]} = \frac{[A]}{[A] + \text{EC}_{50}}
\]

where \( \text{CYP1A} \) and \( \text{CYP1A}_{\text{max}} \) are the amount of CYP1A content measured at inducer concentration \( [A] \) and with 10 nM TCDD, respectively, and \( \text{EC}_{50} \) is the concentration of inducer required to elicit half-maximal CYP1A expression. A modified version of this equation that included a term allowing for nonzero background expression was used to fit data from the cotreatment experiment (Fig. 6).

For AHR binding, total binding (without TCDF and nonspecific binding (with TCDF) were measured as the average of three replicates at each \( ^{[1]}\text{H} \) TCDD concentration. Because the \( ^{[1]}\text{H} \) TCDD concentrations in the total and nonspecific binding treatments were not exactly equal, specific binding is shown as the difference of the total binding at a given concentration and the nonspecific binding at the same concentration as determined from a linear regression of the nonspecific binding data collected. These specific binding values were calculated for illustrative purposes only and were not used for determination of \( K_d \) and \( R_c \). Those values were determined by simultaneous fitting of the data collected to equations describing total and nonspecific binding

\[
TB = \frac{[A] \times [R_c]}{[A] + K_d} - m[A]
\]

\[
NSB = m[A]
\]

where \( TB \) is total binding, \( [A] \) is the concentration of radioligand, \( NSB \) is nonspecific binding, and \( m \) is the slope of the nonspecific binding curve. This method has significant advantages over others, such as Scatchard plots, which can place undue emphasis on a few points of the binding curve (Kenakin, 1999). Specific binding curves were plotted using the Hill–Langmuir isotherm:

\[
[A \cdot R] = \frac{[A] \times [R_c]}{[A] + K_d}
\]

where \( [A \cdot R] \) is the concentration of ligand-receptor complex (i.e., specifically bound ligand). The data were also fit to equations that did not assume a Hill coefficient of 1 (i.e., a lack of cooperative binding), but these showed no statistical improvement, and the Hill coefficients were not significantly different from 1.

Binding inhibition constants \( (K_i) \) were determined by fitting inhibition data from at least three experiments to the Gaddum equation (Gaddum, 1937):

\[
\frac{SB}{R_c} = \frac{[A]}{[A] + K_e \left(1 + \frac{[I]}{K_c}\right)}
\]

where \( SB \) is specific binding and \( [I] \) is the concentration of competitor. Competitive inhibition of \( ^{[1]}\text{H} \) TCDD binding by PCB 105 and antagonism of CYP1A induction by PCB 128 were shown by Schild analysis according to the following regression (Arunlakshana and Schild, 1959):

\[
\log \left( \frac{[I]}{[A]} - 1 \right) = \log [I] - \log K_c
\]

where \( [I] \) is the concentration of ligand required to achieve the same amount of specific binding (or response) in the presence of competitor \( [I] \) that would be achieved by \( [A] \) in the absence of competitor. The ratio \( [A]/[I] \) is called the concentration ratio and is also represented by \( r \). A linear regression of \( \log (r - 1) \) on \( \log [I] \) was performed. The regression supports a mechanism of competitive antagonism if the slope = 1, and in this case alone the intercept provides an independent estimate of \( K_c \).

Stimulus–response coupling for individual AHR agonists was modeled using the operational model of Black and Leff (1983). This assumes a hyperbolic relationship between the amount of ligand-receptor complex and the observed response:
where \( E_m \) is the response observed at agonist concentration \( [A] \), \( E_o \) is the maximal response, and \( K_r \) is the concentration of ligand-receptor complex that gives half-maximal response. \( K_r \) thus represents an efficacy constant analogous to the binding constant \( K_a \). Combining Eqs. (4) and (7) yields:

\[
\frac{E_a}{E_o} = \frac{[R_f] \times [A]}{K_a \times K_r + ([R_f] + K_r) [A]}.
\]

CYP1A induction (i.e., response) data for individual agonists were fit to Eq. (8) using experimentally determined values for \( R_f \) and \( K_a \) (assuming \( K_r = K_a \) for ligands other than TCDD) in order to determine values of \( K_r \). \( K_r \) includes both ligand- and tissue-specific properties, but since these assays were performed in a single cell type, differences among \( K_r \) values are solely ligand-dependent. Relative intrinsic efficacies of the ligands were inferred from AHR binding and CYP1A response data via the efficacy constant, \( K_r \).

Fitting and statistical analyses were performed with SigmaPlot (Jandel Scientific) and JMP In (SAS Institute) software.

RESULTS

CYP1A response to HAH exposure. The induction of CYP1A by HAH in PLHC-1 cells was quantified by its EROD activity and by ELISA. Responses to TCDD, TCDF, and eight PCBs (four non-ortho-, three mono-ortho-, and one di-ortho-substituted) were measured. Representative induction curves are shown in Figs. 1 and 2, and the induction EC50s for all 10 compounds are in Table 1. TCDD, TCDF, all four non-ortho PCBs, and one mono-ortho PCB (156) induced CYP1A protein and catalytic activity, while two other mono-ortho PCBs (105 and 118) and the one di-ortho PCB (128) induced little or no measurable CYP1A.

AHR binding affinities. Binding affinities for the 10 compounds were determined by inhibition of \([^{1}H]TCDD \) binding to the AHR (Fig. 3). Specific binding of \([^{1}H]TCDD \) was measured by a whole-cell filtration method (Dold and Greenlee, 1990). The total, nonspecific, and specific TCDD binding measured in PLHC-1 cells are shown in Fig. 3A. Inhibition curves in Figs. 3B and 3C show the fraction of control \([^{1}H]TCDD \) binding as a function of inhibitor concentration. \( K_r \) values for each compound (Table 1) were determined by simultaneous fitting of three or four such curves from independent experiments, as described under Materials and Methods. \( K_r \) values for the agonists showed the same rank order potency as EROD and CYP1A induction EC50s (Table 1).

Two compounds, PCBs 105 and 128, inhibited TCDD-specific binding but failed to induce EROD or CYP1A, suggesting that they are antagonists in PLHC-1 cells over the range of concentrations used. In order to determine if antagonism by PCB 105 is competitive, binding inhibition was measured at three concentrations of TCDD (Fig. 4A). The resulting Schild plot is shown in Fig. 4B. The slope of the plot is not significantly different from unity, supporting the identification of PCB 105 as a competitive antagonist. The \( K_r \), determined from the intercept of the plot with slope constrained to 1 (2.5 \( \mu M \)) is not significantly different from that determined from the data represented in Fig. 3C (4.6 \( \mu M \); \( p > 0.15 \), t test).

Stimulus–response coupling. The logarithms of EC50 values are plotted against logarithms of \( K_r \) values in Fig. 5A, such that each point represents a single compound. The figure shows that EC50s for CYP1A protein induction increase in a 1:1 relationship with increases in binding affinities, and EC50s are approximately 100-fold lower than \( K_r \) for each compound. This relationship does not hold for EC50s for EROD induction, where the slope of the line is significantly less than 1. This is in agreement with our previous results for a more limited set of compounds showing that EC50s based on EROD induction overestimate relative potencies compared to CYP1A protein induction in the same cells (Hahn et al., 1996; Hestermann et
AHR ligands. This response was measured in the same whole-cell system, it is possible to determine relationships between the two and to calculate relative intrinsic efficacies of the AHR ligands. This was done using the operational model (Black and Leff, 1983), as described under Materials and Methods. The model assumes a hyperbolic stimulus–response relationship, which is consistent with data from other receptor systems and the mechanism of CYP1A induction. Fitted $K_r$ values for the agonists, as well as calculated $R_{50}$ and $R_{95}$ values, are shown in Table 2. $K_r$ represents the amount of receptor-ligand complex required for half-maximal response. These values are on the unit order of magnitude for all full agonists except PCB 156, for which the $K_r$ is $\sim 10$- to 40-fold higher. The $K_r$ values for the other three compounds are at least an order of magnitude greater than that for PCB 156. The $R_{50}$ and $R_{95}$ values are the fraction of receptors that must be occupied to elicit a 50 and 95% response, respectively. Lower values indicate that fewer occupied receptors are necessary for response. Thus, fewer than 30% of the receptors need be occupied for a 95% response to TCDD, while >90% must be occupied for the same response to PCB 156.

The stimulus–response relationship is shown graphically in Fig. 5B, where the fitted constants were used to draw theoretical stimulus–response curves for each agonist. Collectively, the stimulus–response relationships represented in Table 2 and Fig. 5B demonstrate quantitatively what was shown qualitatively in Fig. 5A, that PCB 156 is much less efficient in eliciting a response after binding to the AHR than the other compounds tested. Thus TCDD, TCDF, and the non-ortho-substituted PCBs are high-intrinsic efficacy agonists, and PCB 156 is a low-intrinsic efficacy agonist for the PLHC-1 AHR. Note that all are full agonists, as determined by maximal response, in this system.

**Demonstrating ligand character in a mixture.** These data demonstrate that the compounds tested include representatives of three classes of receptor ligands: high-intrinsic efficacy

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>EROD EC50 (nM)</th>
<th>CYP1A EC50 (nM)</th>
<th>$K_r$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCDD</td>
<td>0.016 ± 0.004</td>
<td>0.015 ± 0.003</td>
<td>0.76 ± 0.25</td>
</tr>
<tr>
<td>TCDF</td>
<td>0.014 ± 0.006</td>
<td>0.032 ± 0.003</td>
<td>1.5 ± 0.60</td>
</tr>
<tr>
<td>PCB 126</td>
<td>0.029 ± 0.004</td>
<td>0.12 ± 0.03</td>
<td>16 ± 7.3</td>
</tr>
<tr>
<td>PCB 81</td>
<td>0.063 ± 0.008</td>
<td>0.19 ± 0.03</td>
<td>29 ± 6.9</td>
</tr>
<tr>
<td>PCB 77</td>
<td>0.73 ± 0.30</td>
<td>14 ± 5</td>
<td>860 ± 420</td>
</tr>
<tr>
<td>PCB 169</td>
<td>1.6 ± 0.43</td>
<td>18 ± 5</td>
<td>2200 ± 1100</td>
</tr>
<tr>
<td>PCB 156</td>
<td>230 ± 61</td>
<td>1900 ± 160</td>
<td>2500 ± 1200</td>
</tr>
<tr>
<td>PCB 118</td>
<td>&gt;50,000$^a$</td>
<td>&gt;50,000$^a$</td>
<td>2900 ± 1300</td>
</tr>
<tr>
<td>PCB 105</td>
<td>ND$^a$</td>
<td>ND</td>
<td>4600 ± 2200</td>
</tr>
<tr>
<td>PCB 128</td>
<td>ND$^a$</td>
<td>ND</td>
<td>6600 ± 3200</td>
</tr>
</tbody>
</table>

Note. All values are mean ± SE of at least three separate determinations, with one such experiment represented in Figs. 1 and 2 for induction and Fig. 3 for binding inhibition.

$^a$ From Hestermann et al., 2000.

$^b$ Minimal induction detected, but insufficient data for determining an EC50.

$^c$ ND, no induction detected.

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**FIG. 2.** CYP1A induction by AHR agonists. Cells were treated as in Fig. 1. ELISA-detected CYP1A protein content was assayed 24 h later. For each compound, the lowest concentration represents treatment with DMSO alone. Points are means ± SE of three wells, and these results are representative of at least three independent experiments. Values are normalized to induction with 10 nM TCDD. The hyperbolic fits to these data are plotted. (A) TCDD, PCB 77, PCB 126, and PCB 169 (Data are from Hestermann et al., 2000.) (B) TCDF, PCB 81, and PCB 156. PCBs 105, 118, and 128 were all nearly or totally inactive in inducing CYP1A.
FIG. 3. Inhibition of [3H]TCDD binding by AHR ligands. Cells were treated with [3H]TCDD in the presence or absence of increasing concentrations of competitors, including a 200-fold excess TCDF treatment to measure nonspecific binding. Specific binding of [3H]TCDD was measured by a whole-cell filtration assay (Dold and Greenlee, 1990). Points are means ± SE of three replicates, and these results are representative of at least three independent experiments. (A) Binding curves for [3H]TCDD in the absence of competitors. The plot through the specific binding points is from Eq. (4), with \( R_s = 103 \) fmol/mg and \( K_d = 0.14 \) nM. Inset shows specific binding on a semilogarithmic plot. Inhibition curves are shown for (B) full agonists and (C) partial agonist and antagonists. Values are fractions of specific [3H]TCDD binding measured in the absence of competitor.

FIG. 4. Competitive inhibition of [3H]TCDD binding by PCB 105. Cells were treated with [3H]TCDD (concentrations in the legend) in the presence of PCB 105 (concentrations indicated on the abscissa). (A) Values are fractions of specific [3H]TCDD binding measured in the absence of competitor and are means ± SE of three replicates. (B) Schild regression of data from A; see Materials and Methods for explanation. The slope of the regression is not significantly different from 1 (\( p > 0.5, t \) test).

agonists (TCDD, TCDF, PCBs 77, 81, 126, and 169), low-intrinsic efficacy agonists (PCB 156 and likely PCB 118), and antagonists (PCBs 105 and 128). Models of receptor action predict that each class of compound should display unique properties when response is measured after cotreatment with a high-intrinsic efficacy agonist such as TCDD (Goldstein et al., 1974, p. 99). A mixture of two high-intrinsic efficacy ligands should produce an additive response. An antagonist should inhibit the response produced by the high-intrinsic efficacy ligand alone. Since a low-intrinsic efficacy agonist has properties of both an agonist and antagonist, it should exhibit concentration-dependent additive and inhibitory effects on the action of the high-intrinsic efficacy ligand.

This cotreatment was done with PCBs 126, 128, and 156 as representatives of each class of ligand (Fig. 6). Cells were treated with a range of concentrations of TCDD in the presence of increasing concentrations of each PCB, and the EC50 for
CYP1A induction by TCDD was measured at each concentration of PCB. For each of the three mixtures, Schild regressions were produced using the fitted EC50s for TCDD at each concentration of PCB (Fig. 7). In such a plot, a high-intrinsic efficacy agonist would be expected to show a slope of 0, a competitive antagonist would be expected to show a slope of 1, and a low-intrinsic efficacy agonist would be expected to show a slope between these two values.

PCB 126 alone induced CYP1A and, in cotreatment, caused only a slight, insignificant increase in EC50s for CYP1A induction by TCDD (Figs. 6A and 7). PCB 128 did not induce CYP1A but did cause a progressive increase in EC50s for CYP1A induction by TCDD (Figs. 6B and 7). PCB 156 induced CYP1A and increased the EC50s for TCDD in cotreatment, although not to the same degree as PCB 128 (Figs. 6C and 7). As predicted, the Schild regression indicates that PCB126 is a high-intrinsic efficacy agonist (slope not significantly different from 0), PCB 156 is a low-intrinsic efficacy agonist (slope significantly different from both 0 and 1), and PCB 128 is a competitive antagonist (slope not significantly different from 1). The y-intercept for the PCB 128 regression predicts a $K_i$ (1.6 μM) that is not significantly different from that determined by ligand binding (6.6 μM; $p > 0.2$, t test).

**DISCUSSION**

This set of experiments represents the first quantitative determination of stimulus–response relationships for AHR ligands in a single system. Structure–activity relationships for both stimulus (receptor binding) and response (CYP1A induction) were determined in intact cells. From such assays, affinities and intrinsic efficacies of ligands were evaluated, allowing the structural parameters that determine agonism to be assessed separately for each of these properties of the ligand–receptor interaction. These data were also used to construct an operational model for AHR–ligand interactions, which has application for risk assessment as well as predicting effects of perturbations to the signaling pathway.

*Interpreting CYP1A induction and competitive binding affinities.* The data presented here demonstrate a 1:1 relationship between AHR binding affinities and CYP1A protein induction.

**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i^*$ (fmol/mg)</th>
<th>$R_{50}$* (%)</th>
<th>$R_{95}$* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCDD</td>
<td>2.0 (0.41)</td>
<td>1.9</td>
<td>28</td>
</tr>
<tr>
<td>TCDF</td>
<td>2.5 (0.72)</td>
<td>2.2</td>
<td>31</td>
</tr>
<tr>
<td>PCB 126</td>
<td>0.95 (0.12)</td>
<td>0.74</td>
<td>13</td>
</tr>
<tr>
<td>PCB 81</td>
<td>0.70 (0.11)</td>
<td>0.97</td>
<td>16</td>
</tr>
<tr>
<td>PCB 77</td>
<td>2.1 (1.0)</td>
<td>1.7</td>
<td>25</td>
</tr>
<tr>
<td>PCB 169</td>
<td>0.95 (0.17)</td>
<td>0.78</td>
<td>14</td>
</tr>
<tr>
<td>PCB 156</td>
<td>28 (3.0)</td>
<td>43</td>
<td>94</td>
</tr>
<tr>
<td>PCB 118</td>
<td>&gt;420'</td>
<td>NA*</td>
<td>NA</td>
</tr>
<tr>
<td>PCB 105</td>
<td>&gt;910'</td>
<td>NA*</td>
<td>NA</td>
</tr>
<tr>
<td>PCB 128</td>
<td>&gt;880'</td>
<td>NA*</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Note.* These values were determined from CYP1A protein induction data as described under Materials and Methods (Eq. (8)).

* $K_i^*$ represents the amount of receptor–ligand complex required for half-maximal response. 

* $R_{50}$ and $R_{95}$ are the fraction of receptors (expressed as a percentage) that must be occupied by the indicated compound for 50 and 95% CYP1A induction, respectively.

* Minimum $K_i$ values were determined by assuming 50% CYP1A induction at 50 μM for PCB 118 and 10% CYP1A induction (the limit of detection) at 50 μM for PCBs 105 and 128.

* These compounds do not produce 50 or 95% maximal tissue responses.
This is due to inhibition of the enzyme activity by the inducing H4IIE cells subsequent analyses and conclusions were drawn from data for affinities and compounds (Gooch induction of here between differences in ortho-substituted PCBs have fits to these data are plotted.

There was also a strong correlation between AHR binding affinities and EC50s for CYP1A-catalyzed EROD induction, which is consistent with earlier studies comparing AHR binding in rat hepatic cytosol and EROD response in vivo and in H4IIE cells (Safe, 1990). However, the 1:1 relationship found here between Ki values and EC50s for induction of CYP1A protein does not hold true with EC50s for EROD induction. This is due to inhibition of the enzyme activity by the inducing compounds (Gooch et al., 1989; Hahn et al., 1993), which lowers EC50s for EROD induction relative to EC50s for CYP1A protein induction (Hahn et al., 1996). For this reason, subsequent analyses and conclusions were drawn from data for induction of CYP1A protein, rather than activity.

Competitive antagonism involves mutually exclusive bind-

![Image](https://via.placeholder.com/150)

**FIG. 6.** Demonstration of ligand intrinsic efficacy by cotreatment. Cells were cotreated with TCDD (concentrations indicated on the abscissa) and varying concentrations of PCB (concentrations indicated in the legend): (A) PCB 126, (B) PCB 128, or (C) PCB 156. The 0.0001 nM concentration of TCDD represents treatment with PCB alone. Points are means ± SE of three wells. Values are normalized to induction with 10 nM TCDD. The hyperbolic fits to these data are plotted.

The correlation exists because TCDD, TCDF, and the non-ortho-substituted PCBs have similar intrinsic efficacies (Table 2), so that differences in AHR binding affinities account for the differences in CYP1A induction potencies among these compounds. However, this same relationship would overestimate Ki,s for lower intrinsic efficacy ligands such as PCBs 156 and 118.

There was also a strong correlation between AHR binding affinities and EC50s for CYP1A-catalyzed EROD induction, which is consistent with earlier studies comparing AHR binding in rat hepatic cytosol and EROD response in vivo and in H4IIE cells (Safe, 1990). However, the 1:1 relationship found here between Ki values and EC50s for induction of CYP1A protein does not hold true with EC50s for EROD induction. This is due to inhibition of the enzyme activity by the inducing compounds (Gooch et al., 1989; Hahn et al., 1993), which lowers EC50s for EROD induction relative to EC50s for CYP1A protein induction (Hahn et al., 1996). For this reason, subsequent analyses and conclusions were drawn from data for induction of CYP1A protein, rather than activity.

Competitive antagonism involves mutually exclusive bind-

![Image](https://via.placeholder.com/150)

**FIG. 7.** Schild regression for cotreatments. The EC50 values from the curves in Fig. 6 were used for regressions. The EC50 value determined in the presence of the highest concentration of each PCB was excluded due to high baseline induction (PCBs 126 and 156) and/or limited solubility (PCBs 128 and 156). The slope of the PCB 126 regression is not significantly different from 0 (p > 0.6) nor is the slope of the PCB 128 regression significantly different from 1 (p > 0.5), while the slope of the PCB 156 regression is significantly greater than 0 and less than 1 (p < 0.01 for both; t test).
TABLE 3
Relative Affinities, Efficacies and Potencies for CYP1A
Protein Induction in PLHC-1 Cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chlorination pattern</th>
<th>Relative affinity</th>
<th>Relative intrinsic efficacy</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCDD</td>
<td>2,3,7,8</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TCDF</td>
<td>2,3,7,8</td>
<td>0.5</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>PCB 126</td>
<td>3,3',4',5</td>
<td>0.05</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td>PCB 81</td>
<td>3,4',5</td>
<td>0.03</td>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td>PCB 77</td>
<td>3,3',4'</td>
<td>0.0009</td>
<td>0.6</td>
<td>0.002</td>
</tr>
<tr>
<td>PCB 169</td>
<td>3,3',4',5,5'</td>
<td>0.0003</td>
<td>2</td>
<td>0.0001</td>
</tr>
<tr>
<td>PCB 156</td>
<td>2,3,3',4',5</td>
<td>0.0003</td>
<td>0.05</td>
<td>0.000001</td>
</tr>
<tr>
<td>PCB 118</td>
<td>2,3',4',5</td>
<td>0.0003</td>
<td>&lt;0.005</td>
<td>&lt;1 X 10^-7</td>
</tr>
<tr>
<td>PCB 105</td>
<td>2,3',3',4'</td>
<td>0.0002</td>
<td>&lt;0.0002</td>
<td>&lt;1 X 10^-4</td>
</tr>
<tr>
<td>PCB 128</td>
<td>2,2',3,3',4,4'</td>
<td>0.0001</td>
<td>&lt;0.002</td>
<td>&lt;1 X 10^-4</td>
</tr>
</tbody>
</table>

Note. Relative affinities, efficacies, and potencies were calculated by dividing the $K_a$, $K_s$, and EC50 for TCDD by that for each compound.

PCBs (Fig. 5A), but also demonstrates that differences in intrinsic efficacy are responsible for differences in induction potency among the ortho-substituted PCBs. Our data provide a mechanism for examples of studies that have noted less than additive interaction for CYP1A induction by mixtures of TCDD and ortho-substituted PCBs both in vivo (Newsted et al., 1995) and in cultured cells (Clemons et al., 1998). The results of this work also have broader applicability. Properties of ligand-receptor interactions and tissue coupling have a large effect on measured relative potencies for any response. Potency depends on both affinity and efficacy, but the toxic equivalency factors (TEF) concept as currently used does not take into account differences in intrinsic efficacy among compounds. This is important because low-intrinsic efficacy compounds will yield less than additive responses in mixtures with high-intrinsic efficacy agonists (Fig. 6). Furthermore, relative potencies from different endpoints and tissues have been used to determine TEFs. Coupling between the receptor and response can be different for these endpoints and tissues, leading to different potencies among measured responses to a single ligand. Thus, a partial agonist for one response or tissue could be a full agonist or an antagonist for another. Therefore, relative potencies are tissue- and endpoint-specific.

Intrinsic efficacy spans a continuum between full agonism and full antagonism. The set of compounds studied here had intrinsic efficacies spanning this range. PCBs 118, 156, and 169 have similar AHR binding affinities (Tables 1 and 3) but produce very different responses. Although there was insufficient response to PCB 118 in this cell type to quantify a stimulus–response relationship, it is clear that the intrinsic efficacy of PCB 118 is less than that of PCB 156, which in turn has a lower intrinsic efficacy than PCB 169. Given that PCBs 105 and 128 have even lower binding affinities, it is possible that they are partial agonists rather than true antagonists and that solubility limitations obscure their nature. However, given that limitation, in this cell type, PCBs 105 and 128 are antagonists in practice, if not in theory.

Stimulus–response modeling for the Ah receptor. AHR binding assays traditionally have been performed using isolated cytosol, a system that preserves only a few of the subsequent signaling events. A few previous studies have approached the question of quantifying AHR ligand intrinsic efficacy using such in vitro systems. The concentration-dependence of AHR binding, DRE mobility shift, and inhibition of DRE mobility shift TCDD-induced were measured using cytosol in studies of substituted ellipticines and flavones (Gasiewicz et al., 1996; Henry et al., 1999). Several of the compounds were also tested for their ability to inhibit TCDD-dependent activation of a DRE-containing reporter construct in mouse hepatoma cells. This system allowed the authors to characterize the compounds' agonistic and/or antagonistic properties, and thereby determine properties of ligand structure that affect those steps. These studies also provide insight to the mechanism of antagonism for this class of compounds (see below). Similarly, a study in cytosol of several dioxin and furan congeners revealed a 10-fold range in receptor binding affinities, but a 100-fold range in EC50 values for DRE mobility shift, (Santostefano et al., 1992). These data suggest differences in intrinsic efficacy among those compounds, related to the ability to promote transformation of the receptor to a DRE-binding form.

While in vitro systems are valuable for determining the mechanism(s) of differences in intrinsic efficacy among ligands, cultured cells allow a more complete assessment of stimulus–response relationships, including tissue coupling. A combination of AHR binding affinities measured in vitro and responses measured in vivo or in cultured cells could potentially be used to classify a compound as an agonist, partial agonist, or antagonist. However, since the concentrations of ligand, receptor, and signaling cofactors vary among the assays, a quantitative stimulus–response model for the tissue cannot be constructed. Our use of whole-cell binding and response assays obviates these complications and thus allows for construction of such a model.

Use of whole cells does introduce complicating factors. Metabolism of AHR ligands can produce artifacts, such as shifts in apparent potency and efficacy, that resemble differences in intrinsic efficacy, as seen by Riddick et al. (1994) in a comparison of TCDD and 3-methylcholanthrene in Hepa-1 cells. Although PCB metabolism has not been measured directly in PLHC-1 cells and data in other fish systems are not abundant, it appears that fish metabolize PCBs very slowly (Hutzinger et al., 1972; Murk et al., 1994; White et al., 1997; Miller et al., 1984; Doucette and Andren, 1988). The highest concentration of PCBs 105 and 128 that could be achieved in the cell culture system used here was 50 μM. In our hands, these PCBs are at or near the limits of their solubility both in the stock solution (10 mM in DMSO) and in the cell culture medium itself (50 μM).
Schlezinger, 1998; Schlezinger et al., 2000), suggesting that differential metabolism was not a factor in the present study. Differences in kinetics of diffusion into the cell are also a potential concern, but the extreme hydrophobicity of these compounds makes such differences unlikely.

The operational stimulus–response model (Black and Leff, 1983) was chosen for this study because the value $K_r$ has a definition that is easily related to the mechanism of AHR signaling. $K_r$ includes both compound- and tissue-specific properties (as well as species-specific properties, for cross-species comparisons). It is a combination of the intrinsic efficacy of the ligand (the ability to activate the receptor to a form that induces a response) and the coupling properties of the cell. Ligand-dependent interactions should not change among tissues within an organism, but the tissue-specific properties can, and, therefore, so might $K_r$ values. This is an important consideration in future efforts to expand modeling to the level of the organism. In our study, the compounds were compared in the same cell type, so tissue-specific properties were constant and differences in $K_r$ values are due solely to differences in the intrinsic efficacies of the ligands.

 Differences in intrinsic efficacy likely depend on ligand-dependent differences in interactions between the AHR and other molecules involved in signaling. Mounting evidence suggests that a mechanism of reduced intrinsic efficacy for many AHR ligands is upstream of DRE binding. The partial agonists α-naphthoflavone (Santostefano et al., 1993), 6-methyl-1,3,8-trichlorodibenzo-furan (Santostefano et al., 1994), and PCB 156 (Petrulis and Bunce, 2000) have been shown in rat hepatic cytosol to inhibit TCDD-induced DRE mobility shift at concentrations lower than those at which the compounds themselves produce such a shift. Neither 2,2',5,5'-PCB (Aarts et al., 1995) nor 2,2',4,4',5,5'-PCB (Petrulis and Bunce, 2000) produce a DRE shift, but both of these di-ortho-substituted PCBs are capable of inhibiting such a shift by TCDD. Several low-intrinsic efficacy AHR ligands produced relatively little DRE binding when compared to high-intrinsic efficacy ligands (Santostefano et al., 1994). Finally, a potent substituted flavone antagonist (but not a structurally similar partial agonist) blocked TCDD-induced translocation of the AHR to the nucleus, as well as subsequent DRE binding (Henry et al., 1999).

The low-intrinsic efficacy of ortho-substituted PCBs suggest an altered ligand–receptor conformation that is less efficient at propagating the signal initiated by ligand binding. Ortho-chlorine substitutions on PCBs hinder the ability of the phenyl rings to assume a coplanar conformation, and this difference in chemical structure may produce an important difference in receptor tertiary structure. Such a change in conformation has been shown for the estrogen receptor. Binding of an antagonist to the estrogen receptor displaces an alpha helix relative to its position when an agonist is bound (Brzozowski et al., 1997), preventing binding of a transcriptional coactivator to the receptor (Shiau et al., 1998). It has been shown recently that the unliganded AHR can interact with ARNT and DREs, but does not induce transcription (Lees and Whitelaw, 1999), suggesting the existence of similar conformation-dependent signaling steps in the AHR pathway. Ligand-dependent differences in AHR conformation also have been suggested by thermodynamic studies (Rosengren et al., 1992), but such differences have not yet been demonstrated directly.

**AHR expression and tissue response.** The value of the operational model lies in its power to predict the effect of perturbations to the tissue. For example, several treatments have been reported to affect expression of the AHR, including phenobarbital (Okey and Vella, 1984), PCB 153 (Denomme et al., 1986), TCDD (Sloop and Lucier, 1987), TGF-β (Dohr et al., 1997), serum withdrawal (Vaziri et al., 1996), and loss of a transcriptional regulator (Zhang et al., 1996). The effect of such changes on tissue response to AHR agonists has been determined in some cases, but not all. A stimulus–response model for the tissue in question should be able to accurately predict the effect of such changes, since receptor concentration, $K_r$, is an element in the model (see Eq. (8) under Materials and Methods).

This model also reveals important aspects of signaling in the absence of perturbation, including the presence of spare receptors. The potential for "spare" or "reserve" AHR has been proposed, beginning with the finding that only a fraction of the agonist-occupied AHR accumulates in the nucleus under conditions of maximal induction of CYP1A1 (Greenlee and Poland, 1979). Modeling of TCDD action has supported the idea that some small fraction of receptors need be occupied to produce a response (Brown et al., 1992, 1994; Andersen et al., 1993). However, quantitative data confirming the existence of spare receptors have been lacking. Analysis of our data using the operational model revealed that only 1–2% of the AHR molecules need be occupied by high-intrinsic efficacy agonists for 50% CYP1A induction (Table 2), demonstrating that PLHC-1 cells have spare receptors for this response. Even after a threefold reduction in receptor content, these compounds should still induce the same maximal level of CYP1A ($R_{50}$ values are less than 33%; Table 2), although higher agonist concentrations would be required. Conversely, there is no receptor reserve for a 95% maximal response to PCB 156, and thus any reduction in AHR content would make this compound a partial agonist for CYP1A induction. However, for another gene or endpoint, differences in coupling could change the fraction of occupied receptors required for a given level of response; eliminating receptor reserve even for high-intrinsic efficacy agonists. The magnitude of receptor reserve is therefore dependent on the agonist, tissue, and response of interest.

In summary, the potency of AHR ligands to induce a response was separated into the properties of affinity and intrinsic efficacy, and the resulting values were used to build a stimulus–response model for AHR signal transduction in PLHC-1 cells. This work is the first study to quantitatively determine intrinsic efficacies of AHR ligands. Stimulus–re-
sponse models can provide useful insights for HAH risk assessment and mechanisms of toxicity across the many endpoints currently under investigation. Expansion of the analyses performed here to other species, tissues, and responses should prove fruitful in studying AHR function and evolution.

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REFERENCES


Schlezinger, J. J. (1998). Involvement of cytochrome P450 1A in the toxicity of aryl hydrocarbon receptor agonists: Alteration of arachidonic acid metab-


