Highly Chlorinated PCBs Inhibit the Human Xenobiotic Response Mediated by the Steroid and Xenobiotic Receptor (SXR)

Michelle M. Tabb,[1] Vladyslav Kholodovych,[2] Felix Grün[1], Changcheng Zhou,[1] William J. Welsh,[2] and Bruce Blumberg[1]

[1]Department of Developmental and Cell Biology, University of California, Irvine, California, USA; [2]University of Medicine & Dentistry of New Jersey–Robert Wood Johnson Medical School and The UMDNJ Informatics Institute, Piscataway, New Jersey USA

Polychlorinated biphenyls (PCBs) are a family of persistent organic contaminants suspected to cause adverse effects in wildlife and humans. In rodents, PCBs bind to the aryl hydrocarbon (AhR) and pregnane X receptors (PXR) inducing the expression of catabolic cytochrome p450 enzymes of the CYP1A and 3A families. We found that certain highly chlorinated PCBs are potent activators of rodent PXR but antagonize its human ortholog, the steroid and xenobiotic receptor (SXR), inhibiting target gene induction. Thus, exposure to PCBs may blunt the human xenobiotic response, inhibiting the detoxification of steroids, bioactive dietary compounds, and xenobiotics normally mediated by SXR. The antagonistic PCBs are among the most stable and abundant in human tissues. These findings have important implications for understanding the biologic effects of PCB exposure and the use of animal models to predict the attendant risk. ***Key words:*** **metabolism, polychlorinated biphenyls, PXR, SXR, xenobiotic.** ***Environ Health Perspect*** **112:163–169 (2004). doi:10.1289/ehp.6560 available via** ***http://dx.doi.org/*** **[Online 24 October 2003]**

Polychlorinated biphenyls (PCBs) are a major health concern. They exhibit many negative biologic effects, they are ubiquitously distributed, and they persist in the environment. Although the manufacture and use of PCBs was banned in the United States in the late 1970s, PCBs were still made and used for many years in other countries. Certain PCBs or PCB mixtures elicit biochemical and toxicologic responses in humans and laboratory animals that mimic those caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin); however, many PCBs, especially more highly chlorinated congeners, do not and many of their nondioxin-like effects remain unknown (Carpenter et al. 2002). PCBs and their metabolites can exert estrogenic effects by binding to the estrogen receptor (ER) (Bonefeld-Jorgensen et al. 2001) or by inhibiting estrogen catabolism (Kester et al. 2000). PCB metabolites have been reported to interfere with transcriptional activation mediated by thyroid hormone (Iwasaki et al. 2002) and androgens (Portigal et al. 2002).

PCBs are lipophilic and accumulate in liver and adipose tissue (Guvenius et al. 2002). Coplanar PCBs that are structurally similar to dioxin and mono-*ortho*-substituted PCBs induce their own metabolism by binding to and activating the aryl hydrocarbon receptor (AhR) (Shimada et al. 2002), which induces CYP1A and CYP1B isozymes (Rendic and Di Carlo 1997). The resulting hydroxylated and methylsulfonyl PCB metabolites covalently bind to DNA, producing double-stranded breaks (Srinivasan et al. 2001). Highly chlorinated PCB congeners are metabolized slowly, if at all, because rapid metabolism requires two adjacent, unsubstituted carbon atoms on the biphenyl ring (Borlakoglu and Wilkins 1993). Thus, highly chlorinated PCBs linger in human tissues, increasing their potential to disrupt normal signaling pathways. Consequently, the ability of a PCB to induce carcinogenesis or mutagenesis or to alter endocrine signaling is strongly associated with its metabolic fate. Dogs and rats metabolize PCBs more readily than monkeys (Matthews and Dedrick 1984), suggesting that the metabolism and biologic activity of individual PCBs may be species specific.

The mammalian xenobiotic response is mediated primarily by two broad-specificity sensors: the orphan nuclear receptors SXR/PXR (human steroid and xenobiotic receptor/rodent pregnane X receptor) (Blumberg et al. 1998; Kliewer et al. 1998) and the constitutive androstane receptor (CAR) (Forman et al. 1998; Xie et al. 2000b; reviewed by Dussault and Forman 2002; Willson and Kliewer 2002). SXR/PXR plays a critical role in the regulation of phase I (cytochrome P450), phase II (conjugating), and phase III (ABC family transporters) detoxifying enzymes, coordinately regulating steroid, drug, and xenobiotic clearance in the liver and intestine (Dussault and Forman 2002; Staudinger et al. 2001; Xie et al. 2000a). SXR/PXR is activated by a diverse group of steroid hormones, dietary compounds (e.g., phytoestrogens), prescription drugs (e.g., taxol, rifampicin), medicinal herbs (e.g., St. John's Wort), and xenobiotics (e.g., organochlorine pesticides) that are all substrates for the SXR-induced enzymes (reviewed by Dussault and Forman 2002; Willson and Kliewer 2002).

Because SXR/PXR exhibits species-specific differences in its response to phytoestrogens, clinically important drugs, and xenobiotics (Blumberg et al. 1998; Jones et al. 2000; Maglich et al. 2002), we infer that the metabolism of some compounds will be correspondingly different between humans and model organisms. To test this possibility, we tested a variety of PCBs to determine their ability to activate human and rodent SXR/PXR. We found that more highly chlorinated PCB congeners were able to activate rodent PXR but not human SXR. These same PCBs were able to directly bind to human SXR and antagonize its activation and target gene induction in primary human hepatocytes and LS180 human colon carcinoma cells. Using SXR transcriptional activation and antagonism data, a predictive molecular model for PCB binding to SXR was formulated, tested, and used to identify additional antagonistic PCBs. The PCBs we identified are among the most persistent and abundant congeners in human tissues and show striking differences in their potential to be metabolized in rodents and humans. PCBs strongly induce their own metabolism in rodents; however, our findings suggest that they antagonize their own metabolism—and that of other xenobiotics, dietary compounds, and endogenous steroids—in humans. Thus, the use of rats to predict the risk of human exposure to these PCBs or mixtures that contain them will likely lead to erroneous conclusions. These findings suggest more broadly that the literature concerning the effects of xenobiotic chemicals and the attendant risks for human and wildlife populations will need to be reevaluated where the

Address correspondence to B. Blumberg, Department of Developmental and Cell Biology, University of California, 5205 McGaugh Hall, Irvine, CA 92697-2300 USA. Telephone: (949) 824-8573. Fax: (949) 824-4709. E-mail: Blumberg@uci.edu

We thank J. Kanno, T. Iguchi, L. Guillette, C. Zilinski, and members of the Blumberg laboratory for critically reading the manuscript, and B. Forman and I. Dussault for plasmids.

Normal human hepatocytes were obtained through the Liver Tissue Procurement and Distribution System, (Pittsburgh, PA) funded by National Institutes of Health (NIH) Contract N01-DK-9-2310. This study was supported by grants from the U.S. Environmental Protection Agency (STAR G1T1 0704) and the National Cancer Institute (CA-87222) to B.B. M.M.T was supported by a training grant from the NIH (Ruth L. Kirschstein NRSA T32 HD-07029).

The authors declare they have no competing financial interests.

Received 2 July 2003; accepted 23 October 2003.

behavior of xenobiotic sensors differs across species. The differential metabolism of drugs, xenobiotics, and dietary compounds mediated by SXR/PXR provides both an explanation and a molecular tool with which to address the often contradictory and controversial literature on the effects of dietary and environmental chemicals on human health.

Materials and Methods

Rat PXR cloning. Human Gal-SXR and mouse Gal-PXR plasmids were described previously (Blumberg et al. 1998); these plasmids contain the yeast Gal4 DNA binding domain fused to the ligand binding domain of SXR or PXR. Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to clone the rat PXR ligand binding domains from liver RNA samples. Briefly, 1 μg total RNA, 100 pmol oligo dT_{18}, and 200 units Superscipt II (Invitrogen, Carlsbad, CA) were incubated according to manufacturer's instructions to generate cDNA. For construction of the Gal4 DBD-PXR ligand binding domain (LBD) fusion plasmid (Gal-PXR), PCR was used to amplify LBD-specific fragments of rat PXR; exonuclease III-mediated subcloning (Li and Evans 1997) was used to clone the fragments between the *Eco*RI and *Bam*HI sites of pCMX-Gal4. Oligos used for PCR were rat forward-5′ TCGCCGGAATTCAAGAAAGAGATGATCATGTC3′, rat reverse 5′-TGGCCAGGATCCTCAGCCGTCCGTGCTG-3′.

Cell culture. Rifampicin (RIF) and pregnenolone 16α-carbonitrile (PCN) were purchased from BioMol (Plymouth Meeting, PA). PCBs were purchased from ChemService, Inc. (West Chester, PA) or NeoSyn Laboratories (New Milford, CT). For ligand treatments, compounds were freshly diluted in DMSO or ethanol prior to addition to cell growth media.

COS7 cells were maintained in phenol red free-Dulbecco modified Eagle medium (DMEM; Cellgro, Kansas City, MO)/10% resin-charcoal stripped fetal bovine serum (FBS; Sigma Chemical Co., St. Louis, MO). Cells were transfected using the calcium phosphate method, and luciferase and β-galactosidase activities were determined as described by Grün et al. (2002). All ligand treatments were performed in triplicate and repeated at least twice.

Northern blotting. Primary human and rat hepatocytes were obtained from In Vitro Technologies (Baltimore, MD) or through the Liver Tissue Procurement and Distribution System program (Pittsburgh, PA). Ligands were added in serum-free hepatocyte incubation media (In Vitro Technologies), and total RNA was prepared using Trizol reagent (Invitrogen). Equal amounts of total RNA (verified by spectrophotometry and ethidium bromide staining) were run on denaturing formaldehyde gels then transferred to Hybond N membrane (Amersham Biosciences, Piscataway, NJ). Probes specific for full-length human *CYP3A4* or rat *CYP3A1* were generated by PCR and labeled with α-(^{32}P) dCTP using the NEBlot kit (New England Biolabs, Beverly, MA). Blots were probed overnight in QuikHyb (Stratagene, Cedar Creek, TX) at 68°C followed by manufacturer's recommended washes.

Quantitative real-time RT-PCR. Primary human hepatocytes or LS180 cells were treated with various ligands for 48 hr and total RNA was prepared as described above. For RT-PCR analysis, 1 μg total RNA was reverse transcribed using Superscript II (Invitrogen). Quantitative real-time RT-PCR (QRT-PCR) was performed using the following primer sets: *CYP3A4*: (F 5′-CTTCATCCAATGGACTGCATAAAT-3′), (R 5′-TCCCAAGTATAACACTCTACACAGACAA-3′); *AhR*: (F 5′-ATTGTGCCGAGTCCCATATC-3′), (R 5′-AAGCAGGCGTGCATTAGACT-3′); *UGT1A1* (UDP-glucuronosyltransferase 1A1): (F 5′-TGCTCATTGCCTTTTCACAG-3), (R 5′-GGGCCTAGGGTAATCCTTCA-3′); *MDR1* (multidrug resistance 1): (F 5′-CCCATCATTGCAATAGCAGG-3′), (R 5′-GAGCATACATATGTTCAAACTTC-3′); glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*): (F 5′-GGCCTCCAAGGAGTAAGACC-3′), (R 5′-AGGGGAGATTCAGTGTGGTG-3′); and the SYBR green PCR kit (Applied Biosystems, Foster City, CA) in a DNA Engine Opticon – Continuous Fluorescence Detection System (MJ Research, Reno, NV). All samples were quantitated by the comparative cycle threshold method for relative quantitation of gene expression, normalized to GAPDH (Livak and Schmittgen 2001).

Ligand binding studies. N-terminal His6-tagged human SXR ligand binding domain was expressed in *Escherichia coli* together with the SRC-1 receptor interaction domain essentially as described (Dussault et al. 2001). Protein was solubilized in 6 M guanidine-HCl, and active protein was refolded from the insoluble pellet fraction using a quick dilution to 0.6 M guanidine-HCl followed by dialysis against binding buffer. Binding assays were performed by coating 96-well nickel chelate FlashPlates (Perkin-Elmer Life Sciences, Boston, MA) with a 10-fold molar excess of protein for 45 min at room temperature in binding buffer (50 mM Hepes, pH 7.4, 200 mM NaCl, 1 M sucrose, 0.1% CHAPS). Unbound protein was removed from wells by washing four times with binding buffer. ^{3}H-SR12813 (Jones et al. 2000) (Amersham BioSciences) was added to a final concentration of 50 nM in each well alone or together with competitor ligands in binding buffer as indicated. Incubation was continued for 3 hr at room temperature. Total counts were measured using a Topcount scintillation counter (Packard, Meriden, CT). Counts remaining after the addition of 10 μM clotrimazole were taken as nonspecific background and subtracted from all wells (Jones et al. 2000). All assays were performed in triplicate and reproduced in independent experiments.

Molecular modeling. All molecular modeling operations were performed on a Silicon Graphics Octane workstation running under the IRIX 6.5 operating system (Silicon Graphics, Inc., Mountain View, CA). The PCB structures in this series were constructed using the Sybyl 6.8 molecular modeling program (Tripos, Inc., St. Louis, MO), followed by initial geometry optimization using the MMFF force field and Gasteiger-Marsili charge set. These structures were then transferred to the Spartan '02 (Wavefunction, Inc., Irvine, CA) for further calculations and visual analysis.

Using Spartan's implementation of MMFF, conformational analysis of each PCB was performed by systematically rotating the central bond between the two rings of the biphenyl skeleton through 360° in 10° increments, followed by energy minimization at each step while constraining the bond's torsion angle. The lowest energy conformer obtained in this way was subjected to further geometry optimization using the AM1 method (Dewer et al. 1985). Previous computational studies (Mulholland et al. 1993) on the conformational properties of PCBs have found that results from AM1 are comparable with experimental values and *ab initio* calculations. The molecular electrostatic potential was calculated and mapped onto the molecular density surface of each PCB for visual analysis, using *ab initio* single-point calculations with the restricted Hartree-Fock wavefunction and 6-31G** basis set (Kong et al. 2000).

Statistics. To determine the potential significance of low level activation of the SXR/PXR from different species, we compared PCB treatments with controls using one-way analysis of variance (ANOVA) using GraphPad Prism software (GraphPad, San Diego, CA). The Bonferonni posttest for multiple comparisons was applied to determine *p*-values.

Results

We surveyed a variety of known or suspected endocrine disrupting chemicals (EDCs) for their ability to activate human, rat, or mouse SXR/PXR and found that PCBs 184 and 196 activated rat and mouse PXR, in accord with published results for mouse PXR (Schuetz et al. 1998). Intriguingly, these PCBs did not activate human SXR (data not shown). To further investigate potential species-specific activation by PCBs, we compared the ability of an initial set of 16 PCBs ranging from trichlorobiphenyl to decachlorobiphenyl to activate mouse and rat PXR and human SXR using

transient transfection assays. COS-7 cells were transfected with a $Gal4_{UAS}$-dependent reporter along with a vector expressing the ligand binding domain of human SXR or mouse or rat PXR linked to the DNA binding domain of yeast Gal4 followed by treatment with various PCBs. PCBs showed distinct species-specific differences in their ability to activate these receptors (Figure 1; PCBs listed in Table 1). PCBs with 5–10 chlorine substituents activated mouse and rat PXR (Figure 1). The completely unsubstituted biphenyl was not able to activate rodent PXR or human SXR. PCBs with ≥ 6 Cl substituents showed some weak activation of human SXR. PCB 201 and PCB 209 yielded almost identical activation in all three species. The human SXR-selective activator RIF and the rodent PXR-selective PCN activated either human SXR or rat and mouse PXR as expected. Similar results were obtained using full-length human SXR or mouse or rat PXR (data not shown). The rank order potency of PCB congeners as rodent PXR activators increased with more extensive *ortho*-chlorination, consistent with published results (Schuetz et al. 1998).

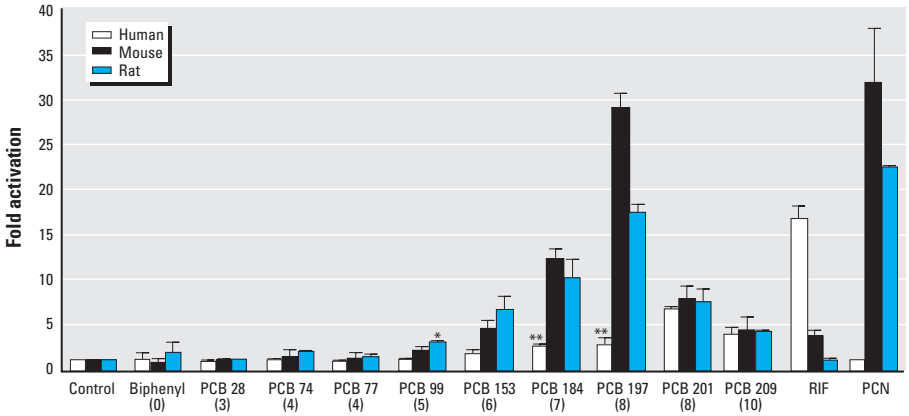

Figure 1. Species-specific activation of SXR/PXR by PCBs in COS-7 cells transiently transfected with human, mouse, or rat Gal-SXR/PXR, tk-MH1004-luc reporter, and CMX-β-galactosidase transfection control plasmids. Cells were treated with 10 μM of the indicated ligands for 24 hr, harvested, and assayed for luciferase and β-galactosidase activity. Data are shown as fold induction of normalized luciferase activity (normalized RLU per optical density of β-galactosidase per minute) compared with control DMSO treatment and represent the mean of triplicate experiments. Error bars indicate SE. Values in parentheses indicate the number of chlorines on each PCB.
*$p < 0.05$ compared to control. **$p < 0.001$ compared to control.

Table 1. PCBs tested and their ability to antagonize human SXR.

IUPAC no.	Name	No. of Cl atoms Total	No. of Cl atoms *Ortho*	Structure	K_i (μM)[a]	*n*
197[b]	2,2′,3,3′,4,4′,6,6′-OctaCB	8	4	N	0.6 ± 0.2	8
184	2,2′,3,4,4′,6,6′-HeptaCB	7	4	N	0.9 ± 0.2	4
183	2,2′,3,4,4′,5′,6-HeptaCB	7	3	N	1.1 ± 0.5	4
145[b]	2,2′,3,4,6,6′-HexaCB	6	4	N	1.3 ± 0.1	4
153	2,2′,4,4′,5,5′-HexaCB	6	2	N	1.9 ± 0.3	4
149[b]	2,2′,3,4′,5′,6-HexaCB	6	3	N	2.0 ± 0.6	4
196	2,2′,3,3′,4,4′,5,6′-OctaCB	8	3	N	2.1 ± 0.6	4
171[b]	2,2′,3,3′,4,4′,6-HeptaCB	7	3	N	2.1 ± 0.3	4
203[b]	2,2′,3,4,4′,5,5′,6-OctaCB	8	3	N	2.4 ± 0.1	4
176[b]	2,2′,3,3′,4,6,6′-HeptaCB	7	4	N	2.4 ± 0.7	4
154[b]	2,2′,4,4′,5,6′-HexaCB	6	3	N	3.1 ± 1.4	4
175[b]	2,2′,3,3′,4,5′,6-HeptaCB	7	3	N	3.1 ± 0.9	4
102[b]	2,2′,4,5,6′-PentaCB	5	3	N	4.6 ± 1.6	4
187	2,2′3,4′,5,5′,6-HeptaCB	6	3	N	6.5 ± 0.4	3
180	2,2′,3,4,4′,5,5′-HeptaCB	6	2	N	8.4 ± 1.0	3
28	2,4,4′-TriCB	3	1	N	9.1 ± 2 4	6
207	2,2′,3,3′,4,4′,5,6,6′-NonaCB	9	4	N	10.8 ± 1.9	3
201[b]	2,2′,3,3′,4′,5,5′,6-OctaCB	8	3	N	16.5 ± 7.9	4
99	2,2′,4,4′,5-PentaCB	5	2	N	24.5 ± 5.3	3
74	2,4,4′,5-TetraCB	4	1	N		
138	2,2′,3,4,4′,5-HexaCB	6	2	N		
209	Decachlorobiphenyl	10	4	N		
–	Biphenyl	0	0			
118	2,3′,4,4′,5-PentaCB	5	1	M		
156	2,3,3′,4,4′,5-HexaCB	6	1	M		
77	3,3′,4,4′-TetraCB	4	0	C		
169	3,3′,4,4′,5,5′-HexaCB	6	0	C		

Abbreviatons: C, coplanar; CB, chlorinated biphenyl; IUPAC, International Union of Pure and Applied Chemistry; M, mono-*ortho*-coplanar; N, noncoplanar.
[a]K_i values were derived from inhibition curves at constant RIF concentrations in the range of 1–10 μM using the Cheng-Prusoff equation; values represent the mean ± SEM calculated from the indicated number of inhibition curves (*n*).
[b]Second set of 10 PCBs, predicted to possess significant antagonist activity.

To confirm that PCBs are species-specific activators, we tested whether they induced SXR/PXR target genes in human and rat primary hepatocytes and in LS180 human colon adenocarcinoma cells. Cells were treated with the human SXR-selective activators bisphenol A or RIF, or the rodent PXR-selective PCN or PCB 184. CYP3A4 (human) or CYP3A1 (rat) mRNA expression was monitored by Northern analysis (Figure 2). RIF and bisphenol A up-regulated steady-state levels of CYP3A4 mRNA in human cells but not CYP3A1 in rat hepatocytes. PCN and PCB 184 up-regulated CYP3A1 mRNA levels in rat hepatocytes but not CYP3A4 in human cells. The inability of PCBs to induce human CYP3A4 is consistent with previous studies (Farin et al. 1994; Schnellmann et al. 1983).

Because the ligand binding domains of SXR and PXR share approximately 80% amino acid identity, we speculated that the PCBs should be able to bind to both receptors. Therefore, we hypothesized that PCBs acting as rodent-selective activators might behave as human-selective antagonists. Accordingly, we next tested each PCB agonist of rodent PXR for its ability to antagonize human SXR in transient transfection assays. Cells were treated with constant amounts of RIF together with increasing PCB concentrations. Inhibition binding constant (K_i) values were derived from inhibition curves and are shown in Table 1. The results showed that highly chlorinated PCBs were able to antagonize activation of

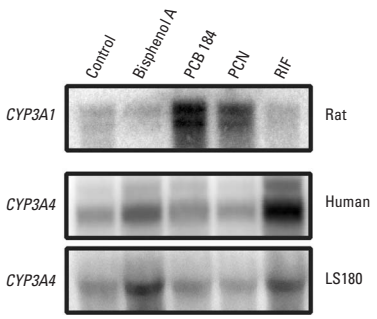

Figure 2. Northern blot analysis showing SXR target gene expression in rat primary hepatocytes, human primary hepatocytes, and LS180 cells. Cells were treated with solvent (control), bisphenol A, PCB 184, PCN, or RIF; then rat primary hepatocyte RNA was probed with *CYP3A1*, and human LS180 and primary hepatocyte RNA probed with *CYP3A4*. See "Materials and Methods" for details.

human SXR, with PCBs 184, 183, and 153 being the most effective antagonists (Table 1).

The biologic activities for the initial set of 16 PCBs were studied using molecular modeling to identify structural patterns associated with those PCBs that antagonize human SXR activation (Table 1). The PCBs were divided into two categories: SXR antagonists ($K_i \leq 9$ μM) and nonantagonists ($K_i > 9$ μM or undetectable). Inspection of the molecular structures revealed distinct substitution patterns that differentiated antagonists from nonantagonists. The most salient feature concerns the total number and specific placement of Cl atoms on the biphenyl skeleton, particularly the number occupying the four *ortho* positions (2, 5, 2′, and 5′). *Ortho* substitution causes the ring–ring torsion angle to rotate farther away from the coplanar conformation in order to minimize steric clashes between *ortho*-Cl atoms on opposite rings. The combination of *meta*, *para* substitution on the same ring with either mono- or di-*ortho* Cl substitution (i.e., 2,3,4,6 or 2,4,5 ring substitution) was associated with the more active compounds (e.g., PCBs 183, 184). As a conceptual aid, we depicted these substitution patterns using a "square" (2,3,4,6-tetrachloro) and a "triangle" (2,4,5-trichloro) benzene ring (Figure 3). The 6 active antagonists in this original set of 16 contain one or more of these motifs (Figure 3; see also Supplemental material online at http://ehp.niehs.nih.gov/members/2003/6560/supp.pdf). In compounds with only a single square or triangle, the other ring contained at least three Cl atoms including at least one *ortho*-Cl atom.

Based on these observations, we inferred the following structural indicators of PCBs that antagonize human SXR: *a*) a Cl-substitution pattern corresponding to 2,3,4,6 ("square") or 2,4,5 ("triangle") on at least one benzene ring; *b*) the "square" pattern imparts greater activity than the "triangle" pattern; *c*) PCBs in which both rings fit one of the patterns should be the most active antagonists; *d*) a ring not fitting either pattern should contain at least one and preferably two *ortho*-Cl atoms and should be *para*-substituted (PCBs 184 vs. 145; 154 vs. 102); and *e*) the PCB molecule should contain at least six Cl atoms overall.

Using these indicators as guides, we predicted that the following PCBs should possess significant antagonistic activity: 102, 145, 149, 154, 171, 175, 176, 197, 201, and 203. Biologic evaluation of this second set of 10 PCBs confirmed our predictions (Table 1). Six of these PCBs were among the 10 most active antagonists, and PCB 197, which fits the "ideal" square–square pattern, was the most active PCB tested (K_i = 0.6 μM; Figures 3 and 4). PCB 201 showed less antagonistic activity than anticipated because it was also a partial agonist (Figure 1).

The SXR antagonism by PCBs in transient transfections suggested that antagonistic PCBs should block human SXR-mediated induction of target genes. SXR is known to regulate genes in all three phases of xenobiotic metabolism, and several other potential target genes such as *AhR* have been identified by microarray analysis (Maglich et al. 2002). We used QRT-PCR analysis to assess *CYP3A4* and *AhR* induction by RIF in primary human hepatocytes and *CYP3A4*, *UGT1A1*, and *MDR1* induction in LS180 cells in the absence or presence of PCBs. As expected, the highly chlorinated PCB 184 and PCB 197, which act as strong antagonists in transfection assays, significantly reduced SXR target gene induction by RIF in both primary human hepatocytes and LS180 cells (Figures 5 and 6). Compounds such as biphenyl and PCB 28, which did not antagonize SXR activation in transient transfection assays, also failed to inhibit the induction of SXR target genes by RIF. A small induction of expression by biphenyl alone and an apparent additive effect of biphenyl plus RIF were observed for AhR expression in primary human hepatocytes, suggesting that biphenyl might be activating AhR to up-regulate its own expression. In the LS180 cells, PCBs were able to antagonize SXR-dependent induction of phase I (CYP3A4), phase II (UGT1A1), and

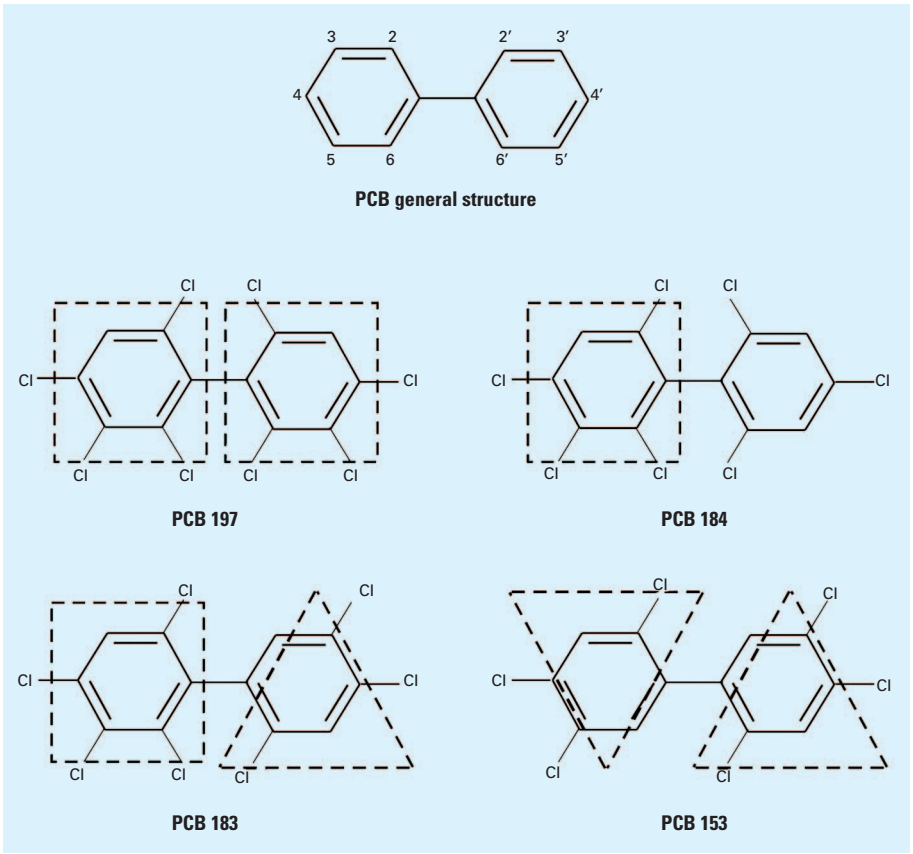

Figure 3. Substitution patterns of PCBs that antagonize human SXR activation. Substitution patterns corresponding to 2, 3, 4, 6 and 2, 4, 5 are shown as squares and triangles, respectively. The strongest antagonists of human SXR contain a "square–square" (PCB 197), "square" plus *ortho*-Cl (PCB 184), "square–triangle" (PCB 183), or "triangle–triangle" (PCB 153) substitution pattern.

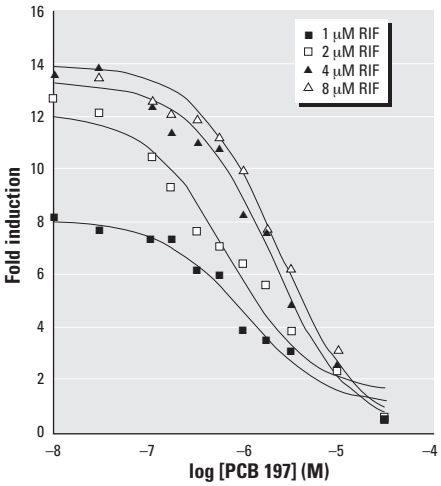

Figure 4. Effect of PCBs on activation of human SXR by rifampicin in COS7 cells transfected with Gal-SXR, tk(MH100)4-luc, and CMX β-galactosidase transfection control plasmids, then treated with RIF in the presence of 1–33 μM PCB 197 for 24 hr. See "Materials and Methods" for details. Data are shown as fold induction of normalized luciferase activity relative to solvent controls. The results shown are from a typical experiment, and data points are the means of triplicates; the SE was < 15%.

phase III (MDR1) metabolic enzymes (Figure 6).

A scintillation proximity *in vitro* ligand-binding assay (Dussault et al. 2001; Jones et al. 2000) was employed to verify that PCBs antagonize human SXR by direct binding rather than through another mechanism. The high-affinity ligand, ^{3}H-SR12813 (K_d = 40 nM) (Jones et al. 2000), directly bound to recombinant human SXR; this binding was specifically antagonized by an excess of unlabeled RIF (Figure 7). PCBs 197, 184, 145, and 153 were all effective competitors (Figure 7). The rank order potency of PCBs as SR12813 competitors closely followed the K_i values of these PCBs for SXR antagonism (Table 1). PCBs that were unable to antagonize human SXR (e.g., biphenyl, PCB 99) and the rodent-selective activator dexamethasone (Moore et al. 2000) did not compete effectively for human SXR binding. We infer from these experiments that antagonistic PCBs bind directly to human SXR to function as antagonists of SXR ligands.

Discussion

Steroid hormones circulating at nanomolar concentrations are essential to normal reproduction and sexual differentiation. Alterations in prenatal levels of sex steroids have been linked to numerous abnormalities, including permanent changes in prostate size and increased risk of ovarian cancer (vom Saal et al. 1997, 1998). SXR is a xenobiotic-sensing nuclear receptor that plays a major role in regulation of steroid, drug, and xenobiotic metabolism [reviewed by Dussault and Forman (2002); Willson and Kliewer (2002)]. Exposure to compounds that activate or block SXR activity could locally alter natural hormone levels by modulating hormone metabolism. Our results show that some PCBs act as agonists of rodent PXR but as antagonists of human SXR, respectively activating or blunting induction of target genes, in both primary human hepatocytes and LS180 human colon carcinoma cells. In agreement with this finding, Easterbrook et al. (2001) have shown that PCBs induce metabolic activity in rat but not human liver microsomes. The data presented above show that rather than inducing metabolic activity in human liver and intestinal cells, exposure to highly chlorinated PCBs inhibits induction of phase I, II, and III metabolic enzymes normally modulated by human SXR (Figures 5 and 6). Thus, PCBs can affect steroid and xenobiotic metabolism, as well as their own metabolism, differently in rodents and humans. Previous studies on the toxicity and long-term effects of exposure to highly chlorinated, nonplanar PCBs in rodents may need to be reevaluated in the light of these new findings.

Coplanar and mono-*ortho*-substituted PCBs initiate their own metabolism by binding to and activating AhR, which in turn induces *CYP1A* and *CYP1B* (Shimada et al. 2002). Most other PCBs are not metabolized efficiently in humans; hence, mechanisms other than AhR activation must mediate their effects (Carpenter et al. 2002). Guvenius et al. (2002) reported that methylsulfonyl and hydroxy PCB metabolites accounted for only 3–26% of the total PCB concentration in human liver and 0.3–0.8% of the total PCBs in adipose tissue. Therefore, many PCBs exist in their native form in human tissues, where they may accumulate because of their inherent lipophilicity. Ligand-induced activation of SXR is normally achieved at micromolar concentrations, and the mixtures of PCBs existing in certain human tissues could approach these levels (Portigal et al. 2002). We note that PCB 153 is one of the most abundant PCBs found in human tissues (Safe 1994) and is not significantly metabolized by human hepatic microsomes (Schnellmann et al. 1983). PCB 153 was among the strongest antagonists of human SXR in our experiments (Table 1). *AhR*, *CYP1A1*, and *CYP1A2* are human SXR target genes (Maglich et al. 2002); therefore, antagonism of SXR-mediated induction of *AhR* and *CYP1A* genes by highly chlorinated PCBs such as PCB 153 may also disrupt the metabolism of the coplanar and mono-*ortho*-substituted PCBs that are normally metabolized through the AhR pathway. Our finding that highly chlorinated PCBs antagonized AhR induction by the SXR activator RIF (Figure 5) is in agreement with this hypothesis. This reasoning suggests that all types of PCBs will be more persistent in humans than in organisms such as rodents, where PCBs activate endogenous PXR and are metabolized. It should be noted that our results are based on experiments using primary human hepatocytes and LS-180 cells. Therefore, it is not impossible that PCBs may be partially

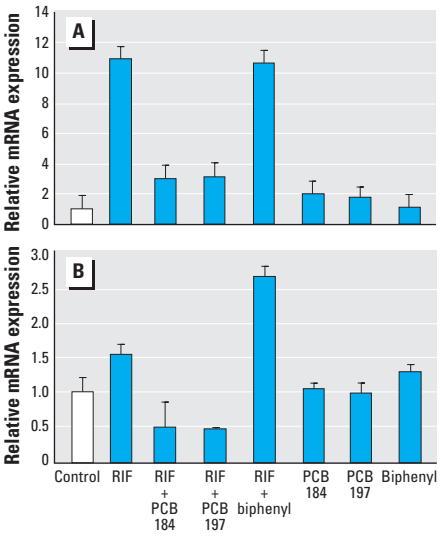

Figure 5. Effect of PCBs on induction of SXR target genes *CYP3A4* (*A*) and *AhR* (*B*) in primary human hepatocytes treated with 10 μM RIF in the absence or presence of 15 μM of the PCBs and expressed relative to solvent controls. Error bars indicate SE. Total RNA was analyzed by QRT-PCR; see "Materials and Methods" for details.

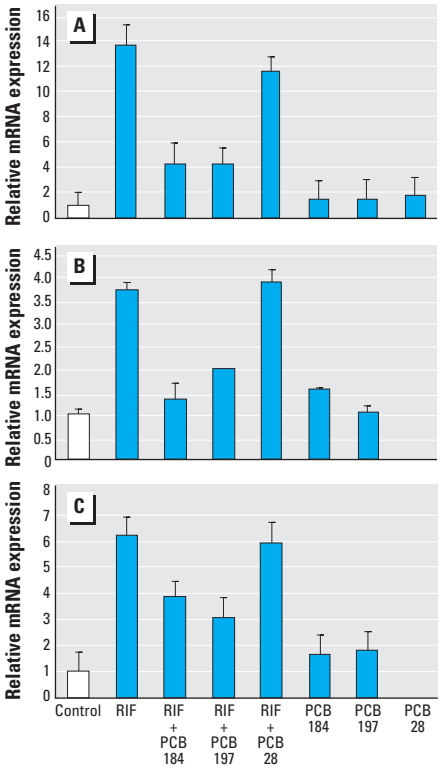

Figure 6. Effect of PCBs on induction of SXR target genes *CYP3A4* (*A*), *UGT1A1* (*B*), and *MDR1* (*C*) in human LS180 colon carcinoma cells treated with 10 μM RIF in the absence or presence of 15 μM of the PCBs and expressed relative to solvent controls. Error bars indicate SE. Total RNA was analyzed by QRT-PCR; see "Materials and Methods" for details.

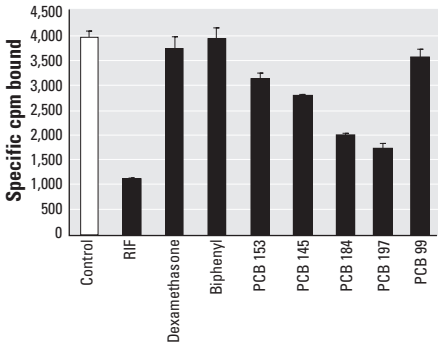

Figure 7. Direct binding of PCBs to human SXR after incubation with 50 nM ^{3}H-SR12813 in the absence or presence of 25 μM of the competitor ligands. Data are expressed as specific total counts per minute (cpm) bound to SXR. Error bars indicate SE. To accurately assess the efficacy of competition, ligand displacement by PCBs should be compared with that of RIF.

metabolized or altered *in vivo*, modifying their ability to bind and antagonize SXR activity.

Loss-of-function mutations in the mouse xenobiotic sensor PXR have been created by targeted gene disruption (Staudinger et al. 2001; Xie et al. 2000a). Such animals are viable and fertile, which could be interpreted to indicate that PXR is not required for normal mammalian physiology. However, treatment of $PXR^{-/-}$ animals with the toxic endogenous bile acid lithocholic acid led to severe liver damage compared with control animals (Staudinger et al. 2001). This suggests that the normal physiologic responses to at least one class of toxicants metabolized via a PXR-dependent pathway are impaired in knockout animals. Although there are currently no published studies examining the effect of chronic toxicant treatment on $PXR^{-/-}$ animals, it would be rather surprising if loss of this critical xenobiotic sensor did not have deleterious consequences. It would be equally surprising if antagonism of SXR did not alter the normal physiologic response to steroids, xenobiotics, and dietary compounds, as has been demonstrated for activation of SXR in promoting drug–drug interactions [reviewed by Dussault and Forman (2002); Willson and Kliewer (2002); Xie and Evans (2001)].

PCB antagonism of human SXR activation has serious implications for human health. PCBs are persistent and ubiquitous environmental contaminants, and humans are exposed to PCBs by eating contaminated food such as beef, poultry, fish, and dairy products, and by infant ingestion of contaminated breast milk (Safe 1994). Extensive epidemiologic evidence has linked PCB exposure to a variety of diseases, developmental problems, and human cancers as well as to adverse reproductive effects in both males and females (Den Hond et al. 2002; Yu et al. 2000). Higher levels of PCBs have been detected in women with benign and malignant breast disease (Güttes et al. 1998), and exposure to PCBs has been linked to increased risk of breast cancer (Dorgan et al. 1999). Exposure to PCBs in food has been linked to delayed brain development, neurobehavioral abnormalities, and reduced IQ (intelligence quotient) in children (Carpenter et al. 2002; Jacobson and Jacobson 1996). A confounding factor is the inability thus far to establish a consistent link between a particular PCB congener and any of these adverse outcomes. Antagonism of SXR activation and inappropriate hormonal modulation by subsets of antagonistic PCBs could lead to many of the human reproductive and developmental effects described. Expression of SXR and its *CYP3A* target genes has been detected in human breast tissue (Huang et al. 1996) and breast cancer cell lines (Dotzlaw et al. 1999) and in rat brain (Schilter et al. 2000). Therefore, it is worth considering the possibility that interference with SXR function caused by members of a group of antagonistic PCBs could contribute to the adverse consequences observed without any requirement for a single congener to be consistently present.

A large number of compounds are known or suspected to have effects on the endocrine system. These endocrine-disrupting compounds may mimic or block the effects of naturally occurring hormones. There is increasing concern about EDCs and their potential for causing adverse health effects in humans and other species. However, there is significant controversy about whether adverse consequences in animal populations can be causally linked to EDC exposure and whether any identified risk can or should be extrapolated to humans. The degree of risk from EDC exposure is variously estimated to range from catastrophic (Colborn et al. 1996) to unproven (Safe 2000) to insignificant (Ames and Gold 2000; Ames et al. 1990); thus it is safe to say that there is disagreement among experts. Further confounding the problem is that humans and wildlife are typically exposed to mixtures of xenobiotic chemicals rather than to a single EDC. Our results demonstrate that highly chlorinated PCBs show striking differences in their potential to be metabolized in rodents and humans. Such PCBs strongly induce xenobiotic metabolism in rodents (Borlakoglu and Wilkins 1993; Easterbrook et al. 2001), whereas they antagonize SXR activation and the expression of phase I, II, and III metabolic enzymes in response to known inducers in primary human hepatocytes (Figure 5) and intestinal cells (Figure 6). Therefore, we infer that these PCBs will inhibit their metabolism and that of other xenobiotics, dietary compounds, and endogenous steroids normally processed through SXR-dependent pathways in humans.

Because rats are the primary mammalian toxicologic model, one cannot escape the conclusion that significant errors will be made when the results of rodent studies are extrapolated to predict human risk, especially when compounds such as these antagonistic PCBs are tested. Partially humanized mice that express human SXR in the livers of transgenic PXR knockout animals (Xie et al. 2000a) may be a better model than rats in some cases. A more appropriate solution would be the use of either a fully humanized rodent that expresses human xenobiotic sensors in all tissues or a model animal that exhibits a response more accurately reflecting human xenobiotic metabolism. The development of a rational public policy for chemical exposure, strongly grounded in sound science, requires the use of model systems that accurately and faithfully predict human outcomes. Xenobiotic-sensing nuclear receptors have an important role to play in this process.

References

Ames BN, Gold LS. 2000. Paracelsus to parascience: the environmental cancer distraction. Mutat Res 447:3–13.

Ames BN, Profet M, Gold LS. 1990. Dietary pesticides (99.99% all natural). Proc Natl Acad Sci USA 87:7777–7781.

Blumberg B, Sabbagh W Jr, Juguilon H, Bolado J Jr, van Meter CM, Ong ES, et al. 1998. SXR, a novel steroid and xenobiotic-sensing nuclear receptor. Genes Dev 12:3195–3205.

Bonefeld-Jorgensen EC, Andersen HR, Rasmussen TH, Vinggaard AM. 2001. Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. Toxicology 158:141–153.

Borlakoglu JT, Wilkins JP. 1993. Metabolism of di-, tri-, tetra-, penta- and hexachlorobiphenyls by hepatic microsomes isolated from control animals and animals treated with Aroclor 1254, a commercial mixture of polychlorinated biphenyls (PCBs). Comp Biochem Physiol C 105:95–106.

Carpenter DO, Arcaro K, Spink DC. 2002. Understanding the human health effects of chemical mixtures. Environ Health Perspect 110(suppl 1):25–42.

Colborn T, Dumanoski D, Myers JP. 1996. Our Stolen Future: Are We Threatening Our Fertility, Intelligence and Survival? New York:Penguin Group.

Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, et al. 2002. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. Environ Health Perspect 110:771–776.

Dewer JS, Zoebisch EG, Healy EF, Stewart JJP. 1985. A new general purpose quantum mechanical molecular model. J Am Chem Soc 107:3902–3909.

Dorgan JF, Brock JW, Rothman N, Needham LL, Miller R, Stephenson HE Jr, et al. 1999. Serum organochlorine pesticides and PCBs and breast cancer risk: results from a prospective analysis (USA). Cancer Causes Control 10:1–11.

Dotzlaw H, Leygue E, Watson P, Murphy LC. 1999. The human orphan receptor PXR messenger RNA is expressed in both normal and neoplastic breast tissue. Clin Cancer Res 5:2103–2107.

Dussault I, Forman BM. 2002. The nuclear receptor PXR: a master regulator of "homeland" defense. Crit Rev Eukaryot Gene Expr 12:53–64.

Dussault I, Lin M, Hollister K, Wang EH, Synold TW, Forman BM. 2001. Peptide mimetic HIV protease inhibitors are ligands for the orphan receptor SXR. J Biol Chem 276:33309–33312.

Easterbrook J, Fackett D, Li AP. 2001. A comparison of Aroclor 1254-induced and uninduced rat liver microsomes to human liver microsomes in phenytoin *O*-deethylation, coumarin 7-hydroxylation, tolbutamide 4-hydroxylation, *S*-mephenytoin 4′-hydroxylation, chloroxazone 6-hydroxylation and testosterone 6β-hydroxylation. Chem Biol Interact 134:243–249.

Farin FM, Pohlman TH, Omiecinski CJ. 1994. Expression of cytochrome P450s and microsomal epoxide hydrolase in primary cultures of human umbilical vein endothelial cells. Toxicol Appl Pharmacol 124:1–9.

Forman BM, Tzameli I, Choi HS, Chen J, Simha D, Seol W, et al. 1998. Androstane metabolites bind to and deactivate the nuclear receptor CAR-β. Nature 395:612–615.

Grün F, Venkatesan RN, Tabb MM, Zhou C, Cao J, Hemmati D, et al. 2002. Benzoate X receptors α and β are pharmacologically distinct and do not function as xenobiotic receptors. J Biol Chem 277:43691–43697.

Güttes S, Failing K, Neumann K, Kleinstein J, Georgii S, Brunn H. 1998. Chlororganic pesticides and polychlorinated biphenyls in breast tissue of women with benign and malignant breast disease. Arch Environ Contam Toxicol 35:140–147.

Guvenius DM, Hassanzadeh P, Bergman A, Noren K. 2002. Metabolites of polychlorinated biphenyls in human liver and adipose tissue. Environ Toxicol Chem 21:2264–2269.

Huang Z, Fasco MJ, Figge HL, Keyomarsi K, Kaminsky LS. 1996. Expression of cytochromes P450 in human breast tissue and tumors. Drug Metab Dispos 24:899–905.

Iwasaki T, Miyazaki W, Takeshita A, Kuroda Y, Koibuchi N. 2002. Polychlorinated biphenyls suppress thyroid hormone-induced transactivation. Biochem Biophys Res Commun 299:384–388.

Jacobson JL, Jacobson SW. 1996. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. N Engl J Med 335:783–789.

Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, et al. 2000. The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. Mol Endocrinol 14:27–39.

Kester MH, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, et al. 2000. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology 141:1897–1900.

Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, et al. 1998. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 92:73–82.

Kong J, White CA, Krylov AI, Sherrill D, Ross D, Adamson TRF, et al. 2000. Q-Chem 2.0: a high-performance ab initio electronic structure program package. J Comput Chem 21:1532–1548.

Li C, Evans RM. 1997. Ligation independent cloning irrespective of restriction site compatibility. Nucleic Acids Res 25:4165–4166.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. Methods 25:402–408.

Maglich JM, Stoltz CM, Goodwin B, Hawkins-Brown D, Moore JT, Kliewer SA. 2002. Nuclear pregnane X receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. Mol Pharmacol 62:638–646.

Matthews HB, Dedrick RL. 1984. Pharmacokinetics of PCBs. Annu Rev Pharmacol Toxicol 24:85–103.

Moore LB, Parks DJ, Jones SA, Bledsoe RK, Consler TG, Stimmel JB, et al. 2000. Orphan nuclear receptors constitutive androstane receptor and pregnane X receptor share xenobiotic and steroid ligands. J Biol Chem 275:15122–15127.

Mulholland JA, Sarofim AF, Rutledge GC. 1993. Semiempirical molecular orbital estimation of the relative stability of polychlorinated biphenyl isomers produced by o-dichlorobenzene pyrolysis. J Phys Chem 97:6890–6896.

Portigal CL, Cowell SP, Fedoruk MN, Butler CM, Rennie PS, Nelson CC. 2002. Polychlorinated biphenyls interfere with androgen-induced transcriptional activation and hormone binding. Toxicol Appl Pharmacol 179:185–194.

Rendic S, Di Carlo FJ. 1997. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. Drug Metab Rev 29:413–580.

Safe SH. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24:87–149.

Safe SH. 2000. Endocrine disruptors and human health—is there a problem? An update. Environ Health Perspect 108:487–493.

Schilter B, Andersen MR, Acharya C, Omiecinski CJ. 2000. Activation of cytochrome P450 gene expression in the rat brain by phenobarbital-like inducers. J Pharmacol Exp Ther 294:916–922.

Schnellmann RG, Putnam CW, Sipes IG. 1983. Metabolism of 2,2′,3,3′,6,6′-hexachlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobiphenyl by human hepatic microsomes. Biochem Pharmacol 32:3233–3239.

Schuetz EG, Brimer C, Schuetz JD. 1998. Environmental xenobiotics and the antihormones cyproterone acetate and spironolactone use the nuclear hormone pregnenolone X receptor to activate the CYP3A23 hormone response element. Mol Pharmacol 54:1113–1117.

Shimada T, Inoue K, Suzuki Y, Kawai T, Azuma E, Nakajima T, et al. 2002. Arylhydrocarbon receptor-dependent induction of liver and lung cytochromes P450 1A1, 1A2, and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in genetically engineered C57BL/6J mice. Carcinogenesis 23:1199–1207.

Srinivasan A, Lehmler HJ, Robertson LW, Ludewig G. 2001. Production of DNA strand breaks in vitro and reactive oxygen species in vitro and in HL-60 cells by PCB metabolites. Toxicol Sci 60:92–102.

Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, et al. 2001. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci USA 98:3369–3374.

vom Saal FS, Cooke PS, Buchanan DL, Palanza P, Thayer KA, Nagel SC, et al. 1998. A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. Toxicol Ind Health 14:239–260.

vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, et al. 1997. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc Natl Acad Sci USA 94:2056–2061.

Willson TM, Kliewer SA. 2002. PXR, CAR and drug metabolism. Nat Rev Drug Discov 1:259–266.

Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, et al. 2000a. Humanized xenobiotic response in mice expressing nuclear receptor SXR. Nature 406:435–439.

Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, et al. 2000b. Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. Genes Dev 14:3014–3023.

Xie W, Evans RM. 2001. Orphan nuclear receptors: the exotics of xenobiotics. J Biol Chem 276:37739–37742.

Yu ML, Guo YL, Hsu CC, Rogan WJ. 2000. Menstruation and reproduction in women with polychlorinated biphenyl (PCB) poisoning: long-term follow-up interviews of the women from the Taiwan Yucheng cohort. Int J Epidemiol 29:672–677.