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 catalytic 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. 2002). 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 (Sriniwasan 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; Kliever 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 Kliever 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 Kliever 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...
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 dT18, and 200 units Superscript II (Invitrogen, Carlsbad, CA) were incubated according to manufacturer’s instructions to generate cDNA. For construction of the Gal4 DDB-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 EcoRI and BamHI sites of pCMX-Gal4. Oligos used for PCR were rat forward-5’-TCGCCGGAATTCAAGAAAGAGATGATCATGTC3´, rat reverse-5’-TCGCCGGAATTCAAGAAAGAGATGATCATGTC3´, rat reverse-5’-TGCCAGGATCCTCTTGAAGCCG-3´, (R 5´-GCCATGTTCAATTGCAGCCCTG-3´), (R 5´-GAGGATCATATGTCAGCCCTG-3´), (R 5´-GAGGATCATATGTCAGCCCTG-3´); glyceraldehyde-3-phosphate dehydrogenase (GAPDH) F 5´-TAGGGGTGTGAACCCGTA-3´, R 5´-CACGGGATCCATCACCA-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. 3H-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 visualization.

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 torsional 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 Bonferroni 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 Gal4UAS-dependent reporter plasmid and a tk-MH1004-luc reporter plasmid. Cells were treated with 10 µM of the indicated ligands for 24 hr, harvested, and assayed for β-galactosidase activity. Data are shown as fold induction of normalized luciferase activity (normalized RLU per optical density of luciferase and β-galactosidase per minute) compared with control DMSO treatment. Error bars indicate SE. Values in parentheses indicate the number of chlorines on each PCB.

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

| IUPAC no. | Name                        | No. of Cl atoms | K_i [µM] | n    |
|-----------|-----------------------------|-----------------|----------|------|
| 197       | 2,2',3,3',4,4',5,6-OctaCB   | 8               | 0.6 ± 0.2| 8    |
| 184       | 2,2',3,4,4',6-HexaCB        | 7               | 0.9 ± 0.2| 4    |
| 183       | 2,2',3,4,4',5,6-HexaCB      | 7               | 1.1 ± 0.5| 4    |
| 145       | 2,2',3,4,4',6-HexaCB        | 6               | 1.3 ± 0.1| 4    |
| 153       | 2,2',4,4',5,5'-HexaCB       | 6               | 1.9 ± 0.3| 3    |
| 149       | 2,2',3,4,4',5,6-HexaCB      | 6               | 2.0 ± 0.6| 4    |
| 196       | 2,2',3,4,4',5,6-OctaCB      | 8               | 2.1 ± 0.1| 4    |
| 171       | 2,2',3,4,4',5,6-OctaCB      | 7               | 2.1 ± 0.2| 3    |
| 203       | 2,2',3,4,4',5,5,6-OctaCB    | 8               | 2.4 ± 0.1| 4    |
| 176       | 2,2',3,4,4',6,6'-HeptaCB    | 7               | 2.4 ± 0.1| 4    |
| 154       | 2,2',4,4',5,6'-HexaCB       | 6               | 3.1 ± 1.4| 4    |
| 175       | 2,2',3,3,3',5,6'-HeptaCB    | 7               | 3.1 ± 0.9| 4    |
| 102       | 2,2',4,5,6'-PentaCB         | 5               | 4.8 ± 1.6| 4    |
| 187       | 2,2',4,5,6'-HeptaCB         | 6               | 5.5 ± 0.4| 3    |
| 180       | 2,2',4,4',5,6'-HeptaCB      | 6               | 8.4 ± 1.0| 3    |
| 28        | 2,4,4'-TricB               | 3               | 9.1 ± 2.4| 6    |
| 207       | 2,2',3,4,4',5,6,6'-NonaCB   | 9               | 10.8 ± 1.9| 3    |
| 201b      | 2,2',3,4,4',5,6-OctaCB      | 8               | 16.5 ± 7.9| 4    |
| 99        | 2,2',4,4',5-PentaCB         | 5               | 24.5 ± 5.3| 3    |
| 74        | 2,4,4'-TetraCB             | 4               | 1       | N    |
| 138       | 2,2',3,4,4',5,6'-HeptaCB    | 6               | 2       | N    |
| 209       | Decachlorobiphenyl          | 10              | 4 ± 2   | N    |
|           | Biphenyl                   | 0               | 0       | N    |
| 118       | 2,2',4,4',5-PentaCB         | 5               | 1       | M    |
| 156       | 2,3,3',4,4',5,6-HexaCB      | 6               | 1       | M    |
| 77        | 2,2',4,4',5,6-HexaCB       | 6               | 0       | C    |
| 189       | 3,3',4,4',5,5,6-HexaCB      | 6               | 0       | C    |

Abbreviations: C, coplanar; CB, chlorinated biphenyl; IUPAC, International Union of Pure and Applied Chemistry; M, mono-ortho-coplanar; N, non-coplanar.

*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).

Second set of 10 PCBs, predicted to possess significant antagonist activity.

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 2. Normal 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 197, PCN, or RIF; then rat primary hepatocyte RNA was probed with CYP3A4, 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 (Ki ≤ 9 µM) and nonantagonists (Ki > 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, 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 (Ki = 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...
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, \(^{3} \text{H}\)-SR12813 (\(K_{d} = 40 \text{nM}\)) (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 (von 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 Kiefer (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 PCBS metabolites accounted for only 3–26% of the total PCBS 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 PCBS 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). PCBS 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 PCBS 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

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**Figure 5.** Effect of PCBS on induction of SXR target genes CYP3A4 (A) and AhR (B) in primary human hepatocytes treated with 10 \(\mu\)M RIF in the absence or presence of 15 \(\mu\)M 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 \(\mu\)M RIF in the absence or presence of 15 \(\mu\)M 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 \(^{3} \text{H}\)-SR12813 in the absence or presence of 25 \(\mu\)M 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 impared 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); Willons and Kiewer (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; Carpenter et al. 2001). 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 (Dortzlaw et al. 1999) and in rat brain (Schütier 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 (Ames and Gold 2000) and 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.

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