

# The Flame Retardants, Polybrominated Diphenyl Ethers, Are Pregnane X Receptor Activators

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Received December 11, 2006; accepted February 16, 2007

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants and are universally present in the environment. An exponential increase in PBDE concentrations in the U.S. population have been reported over the last 3 decades. PBDEs 47 (tetraBDE) and 99 (pentaBDE) are the most commonly detected PBDE congeners in the environment and in human samples. PBDE209 (decaBDE) is the only remaining PBDE flame retardant commercially manufactured in the United States. Several PBDEs are known to induce *cyp3a* in rats, but the mechanism of induction remains unclear. The goal of this study was to clarify the mechanism by which PBDE congeners induce *cyp3a*. Treatment of C57BL6 mice with PBDEs 47, 99, and 209 induced gene expressions of *cyp3a11* and *2b10*, but not *cyp1a1/2*. Because the first two genes are known target genes of pregnane X receptor (PXR), a ligand-activated transcription factor in the nuclear hormone receptor superfamily, we hypothesized that PBDE congeners are PXR activators. Using reporter gene luciferase assays, the present data show that PBDEs 47, 99, and 209 activated PXR and its human counterpart, steroid X receptor, but not aryl hydrocarbon receptor. Furthermore, induction of *cyp3a11* and *2b10* by PBDEs 47, 99, and 209 was markedly suppressed in PXR-knockout mice, indicating that PBDE congeners activate PXR *in vivo*. In summary, our study provides the first evidence that PBDEs are activators for xenobiotic nuclear receptor.

**Key Words:** PBDE; nuclear receptor; PXR, induction; *cyp3a*; *cyp2b*.

The PBDE congeners are used as flame retardants by being incorporated into potentially flammable materials, such as plastics, rubbers, and textiles. The commercial products are produced by bromination of diphenyl oxide and contain mixtures of PBDE congeners (IPCS 1994). PBDEs are released into the environment during their manufacture and disposal and by recycling of plastic materials, such as TV sets and computer

shells (Riess *et al.*, 2000). Human exposure to PBDEs is chronic and is likely via the route of food consumption and inhalation. In the last several decades, increasing use and environmental contamination of PBDEs, coupled with bioaccumulation, has led to a marked increase in PBDE levels in humans. PBDE congeners have been detected in plasma, liver, adipose tissues, and breast milk, suggesting that PBDEs are bioaccumulative and persistent (Covaci *et al.*, 2003; Darnerud *et al.*, 2001; Hardell *et al.*, 1998; Meneses *et al.*, 1999; Ryan, 2000; Sjödin *et al.*, 1999, 2001). The structure for PBDEs is shown in Figure 1. PBDEs 47 (four bromines) and 99 (five bromines) are the predominant congeners detected in humans, whereas PBDE209 (10 bromines) predominates in computer wipes and house dust (Schechter *et al.*, 2003, 2004, 2005a,b, 2006). A wide range of PBDE levels are detected in human samples. For example, the concentrations of PBDE47 in human plasma from the United States range from 10 to 511 ng/g of lipid, with an average of 50 ng/g of lipid (Petreas *et al.*, 2003). In general, human samples from the United States have higher PBDEs than those from Europe or Asia (Darnerud *et al.*, 2001; Schechter *et al.*, 2005b). Of particular concern is the unexpected high levels of PBDEs in U.S. women and milk (Petreas *et al.*, 2003). This is because transporting PBDEs from mother to breast-fed babies can significantly increase the exposure levels of PBDEs to infants.

Numerous studies have shown that PBDEs have adverse effects on laboratory animals. Accumulated evidence show that PBDEs reduce serum total and free thyroxine (T<sub>4</sub>) levels (Fowles *et al.*, 1994; Hallgren *et al.*, 2001; Zhou *et al.*, 2001, 2002), are neurotoxins (Kodavanti and Ward, 2005; Madia *et al.*, 2004), cause hepatic oxidative stress (Fernie *et al.*, 2005), and are labeled as rodent carcinogens (NTP, 1986). These chemicals markedly induce P450 enzyme activities (Fowles *et al.*, 1994; Zhou *et al.*, 2001, 2002), with a recent report showing that PBDEs induce *cyp3a* and *2b* in rats (Sanders *et al.*, 2005); however, the mechanism by which PBDEs induce P450 enzymes remains unknown.

Several mechanisms can be responsible for induction of P450 enzymes, including transcriptional regulation, messenger RNA (mRNA) stabilization, and posttranslational modification

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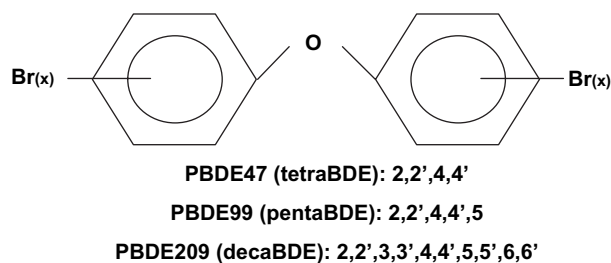


FIG. 1. Chemical structure of PBDEs 47, 99, and 209. DE-71 is a commercial mixture that contains PBDEs 47 and 99, but not 209 (Damerud *et al.*, 2001).

of the proteins (Jaeschke *et al.*, 2002). However, the most common mechanism for inducing *cyp3a* is via activation of PXR, a ligand-activated transcription factor in the nuclear hormone receptor superfamily (Kliewer *et al.*, 2002). PXR can be activated by a wide range of chemicals, including several endogenous compounds, such as pregnanes (Kliewer *et al.*, 1998) and lithocholic acid (Staudinger *et al.*, 2001; Xie *et al.*, 2001), as well as xenobiotics, including rifampicin (antibiotic), tamoxifen (anticancer), troglitazone (anti-type II diabetic), ritonavir (antiviral), and herbal supplements, such as St John's wort (Bertilsson *et al.*, 1998; Blumberg *et al.*, 1998; Jones *et al.*, 2000; Kliewer *et al.*, 1998; Lehmann *et al.*, 1998; Moore *et al.*, 2000; Zhang *et al.*, 1999). The human counterpart for rodent PXR is steroid X receptor (SXR) (Blumberg *et al.*, 1998). There is a clear species difference between rodent PXR and human SXR (Kliewer *et al.*, 2002). For example, PXR is activated by pregnenolone-16 $\alpha$ -carbonitrile (PCN) but not by rifampicin, whereas SXR is activated by rifampicin but not by PCN. In addition, activated PXR and SXR bind to different response elements in the promoters/enhancers of their target genes: the most common response element for PXR is a direct repeat of AGG/TTCA separated by three nucleotides (DR3), whereas the most common response element for SXR is an everted repeat of AGG/TTCA separated by six nucleotides (ER6).

PXR/SXR is crucial to the regulation of metabolism and transport of xenobiotic and endogenous chemicals. Activation of PXR/SXR induces a network of genes important in xenobiotic disposition, such as the uptake transporter *oatp1a4*, phase-I and -II metabolizing enzymes, exemplified by *cyp3a11* and *ugt1a9*, and efflux transporters, such as *mdr1a* and *mrp2* (Abe *et al.*, 1998; Guo *et al.*, 2002; Kliewer *et al.*, 1998; Lehmann *et al.*, 1998). Activation of PXR/SXR results in enhanced elimination of some endogenous chemicals and xenobiotics that are substrates for transporters and/or drug-metabolizing enzymes. However, activation of PXR can also be detrimental because enhanced elimination of endogenous hormones, such as T3/T4, leads to endocrine disruption (Kretschmer and Baldwin, 2005; Zhai *et al.*, 2007; Zhou *et al.*, 2001, 2002). In addition, disruption of the PXR gene in mice reveals severe defects in drug and chemical metabolism and disposition, including the inability to induce *cyp3a11*, the phase-I enzyme that metabolizes over 50% of pharmaceuticals. Furthermore,

TABLE 1  
Animal Groups for P450 Enzyme Induction

Groups	Chemical	Activated nuclear receptor	Dose
1	Corn oil	Vehicle	0.1 ml/10 g of body weight
2	PCB126	AhR	0.09 $\mu$ mol or 0.03 mg/kg/day
3	PCB153	CAR	1.67 $\mu$ mol or 0.6 mg/kg/day
4	PCN	PXR	156 $\mu$ mol or 50 mg/kg/day
5	TCPOBOP	CAR	7.46 $\mu$ mol or 3 mg/kg/day
6	DE-71	Unknown	300 $\mu$ mol or 150 mg/kg/day
7	PBDE47	Unknown	10 $\mu$ mol or 4.9 mg/kg/day
8	PBDE47	Unknown	100 $\mu$ mol or 49 mg/kg/day
9	PBDE99	Unknown	10 $\mu$ mol or 5.7 mg/kg/day
10	PBDE99	Unknown	100 $\mu$ mol or 57mg/kg/day
11	PBDE209	Unknown	10 $\mu$ mol or 9.6 mg/kg/day
12	PBDE209	Unknown	100 $\mu$ mol or 96 mg/kg/day

PXR-knockout mice exhibit dysfunction in apoptosis and response to hepatic oxidative stress, which may affect cell proliferation and tumor formation (Gong *et al.*, 2006; Zucchini *et al.*, 2005).

Thus, the goal of the current study is to test the hypothesis that PBDEs induce P450 *cyp3a* by activation of PXR.

## MATERIALS AND METHODS

**Chemicals.** All chemicals, unless otherwise indicated, were obtained from Sigma (St Louis, MO). The DE-71 mixture was kindly supplied by Great Lakes Chemical (West Lafayette, IN). PBDEs 47 and 99 were obtained from Cerilliant (Round Rock, TX). All reagents were obtained from Fisher Scientific unless otherwise indicated. We obtained [ $^{32}$ P] from PerkinElmer (Wellesley, MA).

**Animals and treatments.** C57BL/6 mice were ordered from the Jackson Laboratories (Bar Harbor, ME). The PXR-knockout mice were originally obtained from Dr Steve Kliewer (University of Texas Southwestern Medical Center) and backcrossed five generations to C57BL/6 background (Guo *et al.*, 2004). All mice were housed in a pathogen-free animal facility under a standard 12-h light:dark cycle with access to regular rodent chow and autoclaved tap water *ad libitum*. All protocols and procedures were approved by the University of Kansas Medical Center Animal Care and Use Committee. Twelve groups of male C57BL/6 mice (10 weeks old,  $n = 3-4$  per group) were treated with PBDE congeners and the appropriate controls. Corn oil was used as the vehicle, and PCB126 was used as the positive control for activation of aryl hydrocarbon receptor (AhR) and induction of *cyp1a1/2*, PCB153, and TCPOBOP (1,4-bis-[2-(3,5-dichloropyridyloxy)]benzene for activation of constitutive androstane receptor (CAR) and induction of *cyp2b10*, and PCN activation of PXR and induction of *cyp3a11* (Table 1). All the chemicals were first dissolved in acetone, followed by addition of corn oil. The remaining acetone was evaporated by speed-vacuum centrifugation. All the chemicals are soluble in acetone, except for PBDE209, which was used as a suspension. The animals were dosed daily by ip injection for 4 days, followed by removal of the liver 24 h after the last injection. Male wild-type or PXR-knockout mice, at 10-12 weeks of age, were treated via ip injection with corn oil, PCN (50 mg/kg/day), TCPOBOP (3 mg/kg/day), PBDEs 47, 99, and 209, at 100  $\mu$ mol/kg/day, for 4 days. Livers were removed and snap-frozen 24 h after the last injection.

**mRNA analysis.** The levels of mRNA were determined by two independent methods: Northern blot analysis and branched DNA (bDNA) assay.

Specifically, total RNA was isolated from the frozen liver using Trizol following the manufacturer's instructions (Invitrogen Inc., Carlsbad, CA). Total RNA concentrations were determined by UV spectrophotometry. For Northern blot analysis, total RNA was analyzed by electrophoresis in 0.22M formaldehyde-containing 1% agarose gel. The sequences for cDNA probes (cyp3a11, 2b10, 1a1/2, and 18s) are available upon request. The Northern blot signal was quantified by using ImageQuant software (GE Healthcare, Piscataway, NJ). The probes were <sup>32</sup>P-labeled using a random primer labeling kit from Roche (Indianapolis, IN). The details for Northern blot analysis and the bDNA can be found in a previous report (Guo *et al.*, 2003; Hartley and Klaassen, 2000).

**Reporter gene assays.** To test activation of mouse PXR, HepG2 cells were transiently transfected with lipofectamine 2000 (Invitrogen) and a cohort of plasmids, including a mammalian expression vector containing cDNA of mouse PXR (pSG5-mPXR), a pGL4-TK vector with three copies of PXR-response element (DR3, AGGTCAnnAGGTC), and pCMV-renilla luciferase vector. Four hours after the transfection, the transfection medium was removed, and the compounds (PCN, PBDEs 47, 99, and 209) that were dissolved in dimethyl sulfoxide at 100mM as stocks were further diluted in the cell culture medium to the desired concentrations. PCN is the positive control that is known to activate rodent PXR. All the compounds are soluble except for PBDE209, which was used as a suspension. Twenty-four hours later, firefly luciferase and renilla luciferase activities were quantified using a Dual Luciferase Kit from Promega (Madison, WI) with a Synergy-HT plate reader (Bio-Tek Instruments, Inc., Winooski, VT). Transfection efficiency was normalized by renilla luciferase activity.

For human SXR, a HepG2 cell line (DPX2) with stable transfection of human SXR and SXR-response element cloned in a luciferase vector was obtained from Puracyp Inc. (Carlsbad, CA). A similar HepG2 cell line, termed DRE, with stable transfection of human AhR and AhR-response element was also obtained from Puracyp, Inc. The construction and validation of the cell lines were reported previously (Yueh *et al.*, 2005a,b). The cells were seeded according to the distributor's instructions, and various testing compounds (PBDEs 47, 99, 209, and rifampicin for SXR and 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) for AhR) at indicated concentrations were added to the culturing medium. The activation of SXR was determined by quantifying the firefly luciferase activity 24 h later, followed by normalization of the luciferase activity by total protein concentrations determined by bicinchoninic acid protein assay (Pierce Inc., Rockford, IL). The reason for using total protein concentration to normalize the response is that all stably transfected cells are derived from a single colony; thus, the transfection efficiency is the same for every cell. However, cell numbers may be different in each well due to human error, which can be normalized by measuring total protein concentration to ensure even cell numbers in each well. Each *in vitro* assay was repeated for at least three times.

**Western blot analysis to determine hepatic cyp3a10 and 2b10 protein levels.** Fifty micrograms of liver protein was loaded onto the sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and hepatic levels of cyp3a11 and 2b10 protein were detected by rabbit anti-mouse cyp3a11 polyclonal antibodies and mouse anti-mouse cyp2b10 monoclonal antibody, respectively, generously provided by Dr Frank Gonzalez at the National Cancer Institute, National Institutes of Health (NIH). The secondary antibody was goat anti-rabbit IgG for cyp3a11 and goat anti-mouse IgG for cyp2b10, both obtained from Sigma. The Western blot signal was quantified by using ImageQuant software (GE Healthcare).

**Statistical analysis.** The data are expressed as mean ± SE. Statistical difference among multiple groups was analyzed by one-way ANOVA analysis, followed by Student-Newman-Keuls test.

## RESULTS

### *PBDE Congeners Are cyp3a11 and 2b10 Inducers in Mice*

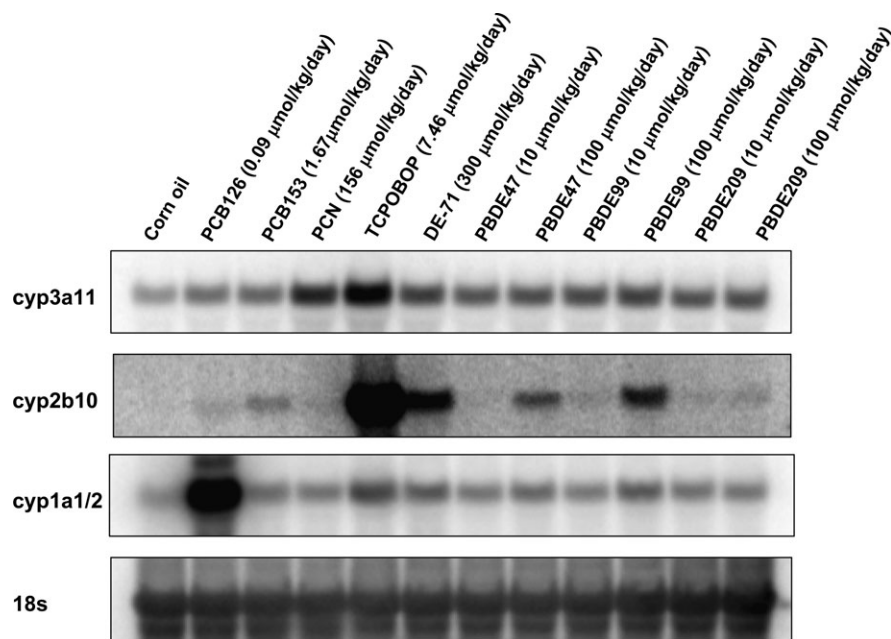
Induction of rat cyp3a and 2b by PBDEs has been demonstrated by Sanders *et al.* (2005). To determine whether

PBDEs induce gene expression of these two enzymes in mice, we treated C57BL/6 young male mice with various chemicals that are known to induce these two enzymes, together with several forms of PBDEs, namely, PBDEs 47, 99, 209, and DE-71, a mixture of PBDEs 47 and 99. Levels of mRNA that are used to assess gene expression were determined and quantified by two independent methods, Northern blot analysis and bDNA assay, shown in Figures 2 and 3, respectively. The fold induction determined from these two methods is summarized in Table 2.

Our data show that all the positive controls induced their corresponding target genes. For example, PCB126 induced 106-fold of cyp1a1/2 by Northern blot analysis and 478-fold by bDNA assay. PCB153 induced fivefold of cyp2b10 by Northern blot and 5.5-fold by bDNA. TCPOBOP induced cyp2b10 more than 2000-fold by Northern blot and 319-fold by bDNA. PCN and TCPOBOP induced twofold of cyp3a11, determined by both Northern blot and bDNA assay. Treatment with DE-71 (containing PBDEs 47 and 99) and PBDEs 47, 99, and 209 induced the mRNA levels of cyp3a11 and 2b10. Specifically, 300 μmol/kg/day of DE-71 and 10 and 100 μmol/kg/day of PBDEs 47, 99, and 209 induced cyp3a11 to a similar degree, four- to fivefold as revealed by Northern blot analysis and twofold by bDNA assay. The cyp2b10 is more ready to be induced compared to cyp3a11, with 110- and 58-fold induction by DE-71 (300 μmol/kg/day), determined by Northern blot analysis and bDNA assay, respectively. PBDEs 47, 99, and 209, at 10 μmol/kg/day, induced cyp2b10 eleven-, four-, and three-fold, respectively, determined by Northern blot analysis and two-, five-, and fourfold, respectively, by bDNA assay. At 100 μmol/kg/day, these PBDE congeners induced cyp2b10 to a higher level with 42-, 74-, and 5-fold induction, respectively, by Northern blot analysis and 22-, 37-, and 7-fold induction, respectively, by bDNA assay. The levels of cyp1a1/2 were induced by all the chemicals treated when determined by Northern blot analysis. Because Northern blot analysis is not as specific as bDNA assay, mostly due to cross-react of the Northern blot probes to transcripts with similar sequences, we also measured cyp1a1/2 mRNA levels by bDNA assay, which clearly shows that except for PCB126 and PCB153, none of the chemicals induced cyp1a1/2, including PBDE congeners.

### *PBDEs Are PXR/SXR Activators In Vitro*

Most cyp3a11 and 2b10 inducers are activators for PXR, so we tested whether PBDEs are activators for mouse PXR and human SXR. Shown in Figure 4, at lower concentrations (100nM and 1μM), only PBDE99 activated PXR. At higher concentrations (10μM), PBDEs 47 and 99 activated mouse PXR to a similar degree, and at the highest concentration tested (100μM), PBDE209 also activated PXR. Compared to the known PXR activator, PCN, which activates PXR at relatively higher concentrations (10 and 100μM), PBDEs 47 and 99 activate PXR at lower concentrations (1μM for PBDE47 and



**FIG. 2.** Northern blot analysis of hepatic *cyp3a11*, *cyp2b10*, and *cyp1a1/2* mRNA levels after treatment of mice with various chemicals shown in Table 1. The 12 lanes are the pooled total RNA from the 12 groups of mice. Specifically, lane 1 is for corn oil, lane 2 for PCB126, lane 3 for PCB153, lane 4 for PCN, lane 5 for TCPOBOP, lane 6 for DE-71, lanes 7 and 8 for PBDE47 at 10 and 100  $\mu\text{mol/kg/day}$ , respectively, lanes 9 and 10 for PBDE99 at 10 and 100  $\mu\text{mol/kg/day}$ , respectively, and lanes 11 and 12 for PBDE209 at 10 and 100  $\mu\text{mol/kg/day}$ , respectively. The internal loading control is the 18s ribosomal RNA because several housekeeping genes, such as GAPDH, may be induced by microsomal enzyme inducers. The fold induction is summarized in Table 2.

100nM and 1 $\mu\text{M}$  for PBDE99). However, at higher concentration (10 and 100 $\mu\text{M}$ ), PCN is stronger than PBDE congeners in activating PXR. Activation of human SXR is shown in Figure 5A. All three PBDEs activated human SXR dose dependently. In contrast to activating mouse PXR, the potency of PBDEs 47 and 99 is similar in activating human SXR, followed by PBDE209. In addition, one striking observation for these PBDE congeners is that they activate SXR to a similar degree compared to a known SXR potent ligand, rifampicin. We also tested whether PBDEs activated AhR *in vitro* in a HepG2 cell line stably transfected with human AhR cDNA and AhR-response element in a luciferase vector (DRE cell line). The results show that at concentrations that activated SXR, these three PBDEs activated AhR to a much less degree than the classical AhR activator, TCDD (Fig. 5B). Therefore, the *in vitro* experiments show that PBDEs are mouse PXR and human SXR activators, but are very weak or do not activate human AhR.

#### PBDEs Are PXR Activators In Vivo

After determining that PBDEs are PXR/SXR activators *in vitro*, we tested whether PBDEs activate PXR *in vivo* by using PXR-knockout mice with pure C57BL/6 background. As shown in Figure 6A and 6C, treatment of wild-type mice with PBDEs 47, 99, and 209 induced *cyp3a11* mRNA and protein, compared to corn oil-treated group. However, the induction of

*cyp3a11* mRNA and protein in the PXR-knockout mice was markedly suppressed when comparing the levels of *cyp3a11* between corn oil- and PBDEs-treated groups, but not completely diminished. The induction of *cyp2b10* mRNA by PBDEs in PXR-knockout mice was suppressed, and the induction of *cyp2b10* protein was abolished (Fig. 6B and 6C).

## DISCUSSION

The current study provides convincing evidence that PBDEs induce *cyp3a11* and *2b10*, but not *cyp1a1/2* in mouse livers. We also provide the first evidence that PBDEs activate both PXR and SXR *in vitro*. In addition, induction by PBDEs of the genes encoding these enzymes is suppressed in PXR-knockout mice compared to wild-type mice. This study is important because for the first time the molecular mechanism by which PBDEs induce P450 enzymes is elucidated. In addition, because little information regarding human exposure and toxicity of PBDEs is available, our study helps to provide a scientific basis for extrapolating data from rodents into humans by comparing the data obtained *in vitro* from interaction of PBDEs with both rodent and human xenobiotic receptors. In this regard, knowledge obtained from this study will aid in understanding and future prediction of PBDE toxicities in humans.

The present study not only shows that PBDEs induce mouse *cyp3a11* and *2b10* but also indicates that the effectiveness of

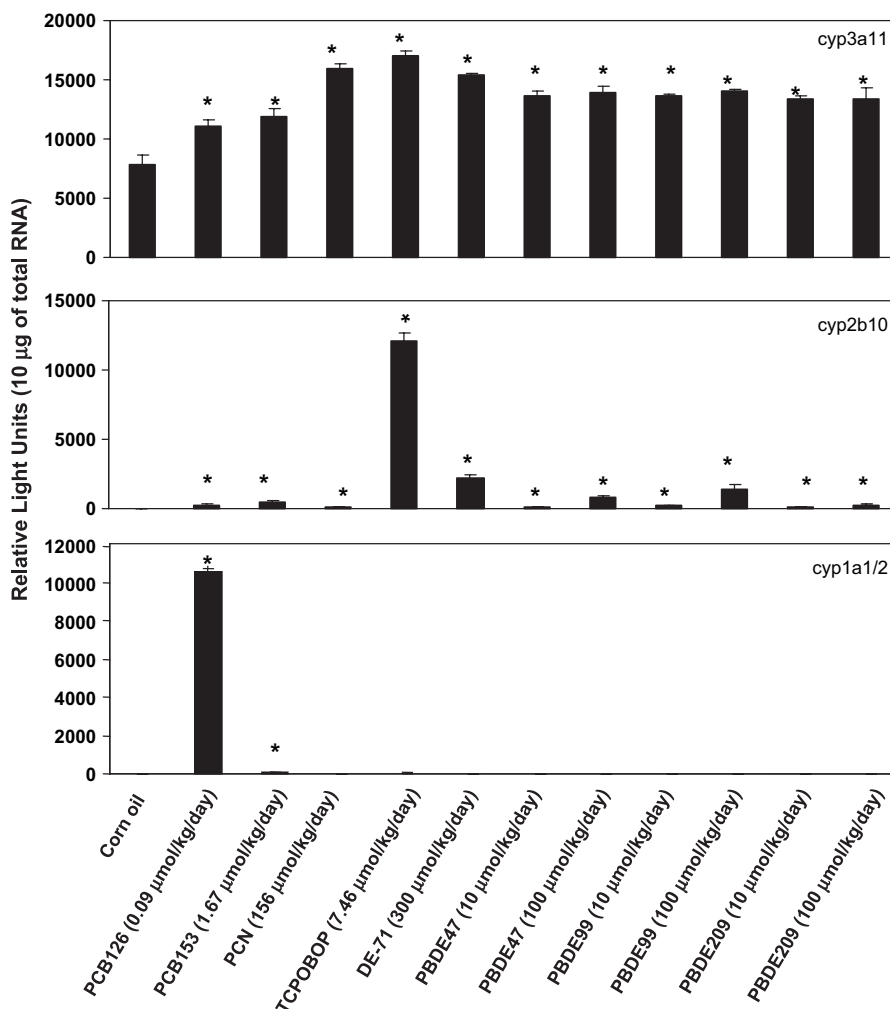


FIG. 3. Levels of hepatic cyp3a11, cyp2b10, and cyp1a1/2 mRNA determined by bDNA assay. Total RNA was isolated from livers of mice treated with the chemicals shown in Table 1. The mRNA levels of cyp3a11, cyp2b10, and cyp1a1/2 were determined by bDNA assay. The asterisk indicates that  $p$  value is  $< 0.05$  comparing chemical- and corn oil-treated groups. The fold induction of these three genes is summarized in Table 2.

PBDEs in inducing the genes encoding these two enzymes is different. While the ability of PBDEs 47, 99, and 209 in inducing mouse cyp3a11 is similar regardless of the degree of bromination and dosage, they are different in inducing cyp2b10. PBDE99 is the strongest in inducing cyp2b10, followed by PBDEs 47 and 209. We think that the difference of PBDEs in inducing these two genes relies on the basal levels of these two P450 enzymes as well as their ability to respond to inducers. The basal gene expression level of cyp3a10 is high, which makes it relatively easier to reach the saturation point where cyp3a is no longer responsive to inducers; thus, the fold induction of this enzyme is relatively low, and even the total amount of protein is abundant. In contrast, the basal level of cyp2b is very low, and thus, the fold induction can be high. This may also be the reason that treatment with three times higher concentration of DE-71, a mixture of PBDEs 47 and 99, did not result in three-fold higher induction of cyp3a11 and 2b10 compared to PBDEs 47 and 99.

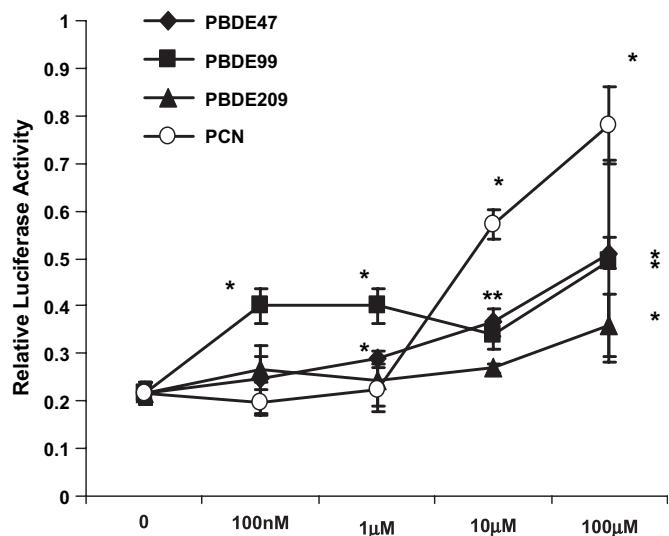
Although structurally similar to PCBs, PBDEs appear unlikely to be inducers for cyp1a1/2 in mice. We used two methods to determine the mRNA levels of cyp1a1/2 after treatment with various PBDEs and PCB126, a chemical known to activate AhR and to induce cyp1a1/2. The results obtained from Northern blot analysis showed an induction of cyp1a1/2 by all the compounds tested. However, Northern blot analysis is not as specific as bDNA assay due to the fact that probes used in Northern blot are fragment of cDNA, which can cross-react with other transcripts with sequence similar to cyp1a1/2. Therefore, we also determined cyp1a1/2 mRNA levels by bDNA assay that specifically detects a single-gene transcript. The results obtained from bDNA assay clearly showed that PBDEs are not cyp1a1/2 inducers. This is consistent with the studies reported previously that PBDEs are not AhR activators and do not induce cyp1a1/2 (Peters *et al.*, 2004; Sanders *et al.*, 2005).

Our study provides the first evidence that PBDEs are activators for xenobiotic nuclear receptor, PXR. The induction of

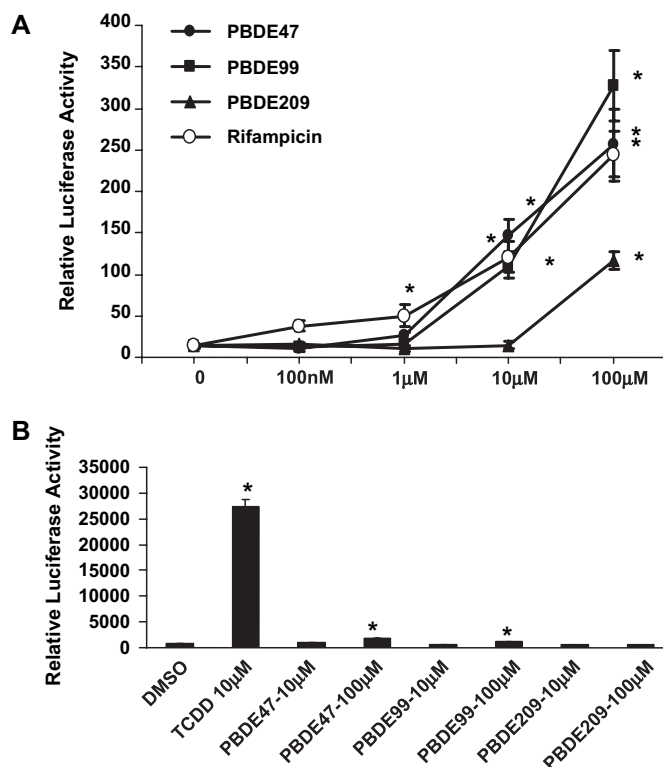
**TABLE 2**  
Fold Induction of Hepatic *cyp3a11*, *cyp2b10*, and *cyp1a1/2*  
Following PBDE Treatment (obtained from Northern  
blot analysis and bDNA assay)

Groups	Chemicals ( $\mu\text{mol/kg/day}$ )	<i>cyp3a11</i>		<i>cyp2b10</i>		<i>cyp1a1/2</i>	
		Northern blot	bDNA	Northern blot	bDNA	Northern blot	bDNA
1	Corn oil	1	1	1	1	1	1
2	PCB126 (0.09)	3	1.4	5	5.5	106	477.7
3	PCB153 (1.67)	4	1.5	21	12.3	6	3.1
4	PCN (156)	10	2.0	4	3.8	9	1.0
5	TCPOBOP (7.46)	17	2.2	2139	319.4	37	1.8
6	DE-71 (300)	5	2.0	110	57.5	17	1.0
7	PBDE47 (10)	5	1.8	11	2.1	10	0.7
8	PBDE47 (100)	5	1.8	42	21.9	16	0.8
9	PBDE99 (10)	5	1.8	4	5.1	9	1.0
10	PBDE99 (100)	5	1.8	74	36.9	16	0.9
11	PBDE209 (10)	4	1.7	3	3.6	9	0.5
12	PBDE209 (100)	4	1.7	5	7.0	6	0.8

Note. The fold induction was obtained by comparing mRNA levels between corn oil- and chemical-treated mice. Northern blot was quantified by ImageQuant computer software.



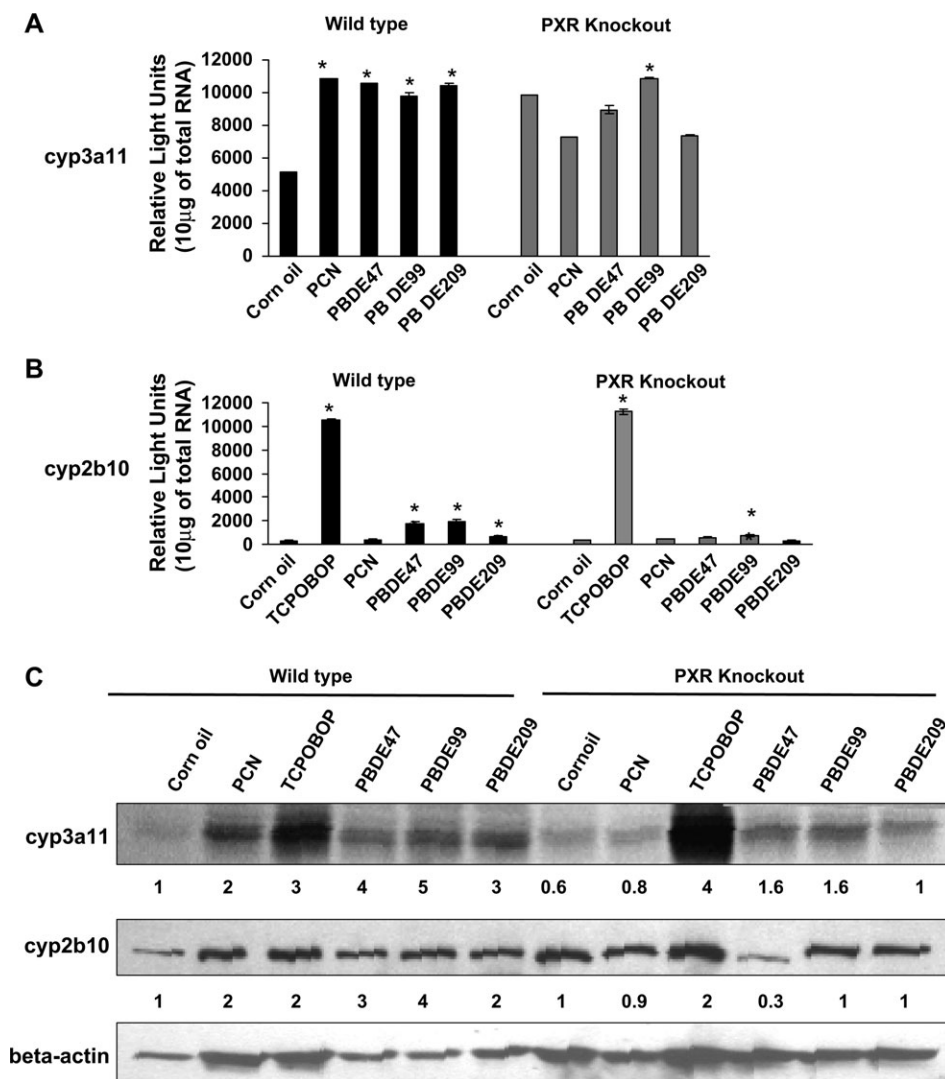
**FIG. 4.** *In vitro* activation of mouse PXR by PBDEs 47, 99, and 209. HepG2 cells were transiently transfected with mouse PXR expression vector (pSG5-mPXR), three copies of PXR-response element DR3 in a firefly luciferase vector pGL4-TK, and pCMV-renilla luciferase vector that serves as the control for transfection efficiency. Twenty-four hours following addition of PCN or PBDEs at indicated concentrations, firefly and renilla luciferase activities were quantified, and the transfection efficiency was normalized by comparing firefly and renilla luciferase activities. The asterisk indicates that  $p$  value is  $< 0.05$  comparing dimethyl sulfoxide-treated cells (concentration 0). This experiment was repeated three times with similar results.



**FIG. 5.** *In vitro* activation of human SXR and AhR by PBDEs 47, 99, and 209. The DPX2 cells with stably transfected human SXR and SXR-response element in a firefly luciferase vector or the DRE cells with stably transfected human AhR and AhR-response element in a firefly luciferase vector were treated with various concentrations of PBDEs and rifampicin, or TCDD (10 $\mu\text{M}$ ). Twenty-four hours following addition of PBDEs at the indicated concentrations, luciferase activity was quantified, and the activation of receptors was normalized by total protein concentration. Panel (A) is for SXR and panel (B) for AhR. Asterisks indicate that  $p$  value is  $< 0.05$  comparing chemical- and dimethyl sulfoxide-treated cells (concentration 0). This experiment was repeated three times with similar results.

*cyp3a11* and *2b10* by PBDE congeners highly suggests that PBDEs may be activators for xenobiotic nuclear receptor, namely PXR and/or CAR. In the current study, we focus on investigating the interaction between PBDEs and PXR. Our *in vitro* studies show that PBDEs 47, 99, and 209 are activators of both mouse PXR and human SXR. Based on our data, the potency for these three PBDEs is similar in activating mouse PXR, which is consistent with gene expression data that induction of *cyp3a11* by these three PBDEs is similar regardless of the magnitude of bromination. However, PBDEs 47 and 99 activated SXR at a much lower concentration than PBDE209, indicating that they are more potent activators for SXR than PBDE209. The activation of PXR/SXR by PBDEs is likely the mechanism for induction of *cyp3a11* and *2b10 in vivo*, which is further confirmed by the results gathered from the PXR-knockout mice, showing that disruption of PXR abolished the induction markedly. PBDEs may induce *cyp3a11* and *2b10* via activating CAR. This is because PBDEs induce





**FIG. 6.** Hepatic levels of cyp3a11 and cyp2b10 mRNA and protein in wild-type and PXR-knockout mice after treatment with PBDEs. Male wild-type or PXR-knockout mice,  $n = 4$ , at 10–12 weeks of age were ip injected with corn oil, PCN (50 mg/kg/day), TCPOBOP (3 mg/kg/day), PBDEs 47, 99, and 209, at 100  $\mu$ mol/kg/day, for 4 days. Twenty-four hours after the last injection, the livers were removed and total RNA were isolated. The mRNA levels of cyp3a11 (panel A) and cyp2b10 (panel B) were determined by bDNA assay. The protein levels of these two enzymes, and beta-actin that is the loading control, from pooled samples were determined by Western blot analysis (panel C). The Western blot signal was quantified by using ImageQuant software (GE Healthcare). The fold induction was indicated below each blot. For panels (A) and (B), the statistical comparison was made between corn oil- and chemical-treated groups with the same genotype. The asterisk indicates  $p < 0.05$  between corn oil- and chemical-treated groups.

cyp2b10, a classical target gene for not only PXR but also for CAR. In addition, treatment with the PBDEs in the PXR-knockout mice induced cyp3a11 and 2b10 to a less degree, but the induction was not completely abolished. This suggests that factors other than PXR are also involved in induction of these P450 enzymes. Furthermore, we also cannot exclude other factors, independent of the xenobiotic nuclear receptors, that might be involved for induction of cyp3a11 and 2b10. These two possibilities will be further tested by using CAR- and PXR/CAR double-knockout mice in the future studies.

In summary, we have demonstrated that PBDEs, the widely used brominated flame retardants, are inducers of P450 enzymes, cyp3a11 and 2b10 in mice. The mechanism for this

induction is, at least partly, due to activation of the xenobiotic nuclear receptor, PXR. PBDEs 47, 99, and 209 also activate human SXR, indicating that PBDEs may elicit toxic effects in humans by similar mechanisms as in rodents.

#### ACKNOWLEDGMENTS

We acknowledge Ms Noriko Esterly for her excellent technical support. We are grateful for the critical reagents, cell lines, and manuscript review from Drs Michael Sanders (National Institute of Environmental Health Sciences), Kyle Kramer, and Judy Raucy (Puracyp Inc.). We appreciate the funding from KUMC Endowment fund, K12 HD052027 (NIH/NICHD), P20 RR015563 (NIH), and P20 RR021940 (NIH) that made this study possible.

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