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Interactions of Polybrominated Diphenyl Ethers with the Aryl Hydrocarbon Receptor Pathway

A. K. Peters[*,1], **S. Nijmeijer**[*], **K. Gradin**[†], **M. Backlund**[‡], **Å. Bergman**[§], **L. Poellinger**[†], **M. S. Denison**[¶], and **M. Van den Berg**[*]

[*] Institute for Risk Assessment Sciences, Utrecht University, PO Box 80176, 3508 TD Utrecht, The Netherlands [†] Department of Cell and Molecular Biology, Institute of Environmental Medicine, Karolinska Institute, SE-17177, Stockholm, Sweden [‡] Division of Molecular Toxicology, Institute of Environmental Medicine, Karolinska Institute, SE-17177, Stockholm, Sweden [§] Department of Environmental Chemistry, Wallenberg Laboratory, Stockholm University, SE-10691 Stockholm, Sweden [¶] Department of Environmental Toxicology, Meyer Hall, University of California—Davis, One Shields Avenue, Davis, California 95616

Abstract

Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants that have been in use as additives in various consumer products. Structural similarities of PBDEs with other polyhalogenated aromatic hydrocarbons that show affinity for the aryl hydrocarbon receptor (AhR), such as some polychlorinated biphenyls, raised concerns about their possible dioxin-like properties. We studied the ability of environmentally relevant PBDEs (BDE-47, -99, -100, -153, -154, and -183) and the “planar” congener BDE-77 to bind and/or activate the AhR in stably transfected rodent hepatoma cell lines with an AhR-responsive enhanced green fluorescent protein (AhR-EGFP) reporter gene (H1G1.1c3 mouse and H4G1.1c2 rat hepatoma). 7-Ethoxyresorufin-O-dee-thylation (EROD) was used as a marker for CYP1A1 activity. Dose- and bromination-specific inhibition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced responses was measured by their ability to inhibit the induction of AhR-EGFP expression and EROD activity. Individual exposure to these PBDEs did not result in any increase in induction of AhR-EGFP or CYP1A1 activity. The lower brominated PBDEs showed the strongest inhibitory effect on TCDD-induced activities in both cell lines. While the highest brominated PBDE tested, BDE-183, inhibited EROD activity, it did not affect the induction of AhR-EGFP expression. Similar findings were observed after exposing stably transfected human hepatoma (xenobiotic response element [XRE]–HepG2) cells to these PBDEs, resulting in a small but statically significant agonistic effect on XRE-driven luciferase activity. Co-exposure with TCDD resulted again in antagonistic effects, confirming that the inhibitory effect of these PBDEs on TCDD-induced responses was not only due to direct interaction at receptor level but also at DNA-binding level. This antagonism was confirmed for BDE-99 in HepG2 cells transiently transfected with a Gal4-AhR construct and the corresponding Gal4-Luc reporter gene. In addition, a chromatin immunoprecipitation assay further confirmed that BDE-99 could bind to the AhR and activate the AhR nuclear translocation and dioxin responsive element (DRE) binding in the context of the CYP1A1 promoter. However, the transactivation function of the BDE-99-activated AhR seems to be very weak. These combined results suggest that PBDEs do bind but not activate the AhR–AhR nuclear translocator protein–XRE complex.

[1]To whom correspondence should be addressed at Institute for Risk Assessment Sciences, Utrecht University, Yalelaan 2, PO Box 80176, 3508 TD Utrecht, the Netherlands. Fax: +31-30-2535077. l.peters@iras.uu.nl.

Keywords

aryl hydrocarbon receptor; PBDE; EROD; AhR-EGFP; XRE

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are a group of brominated flame retardants and have been in use as additives in various consumer products since the 1970's. Although PBDEs are beneficial in that they reduce the chances of ignition and burning of materials, they have the ability to leach out of the polymer in which they are present. There are three major commercial PBDE mixtures that are or have been produced, differing in their degree of bromination; the penta-, octa-, and deca-mix. Some PBDE congeners have been found to be lipophilic with log Kow values varying from 6 for penta- to 10 for deca-BDE (Watanabe and Tatsukawa, 1989). Accordingly, levels of PBDEs have been detected world wide in many matrices, for example, in fish, wildlife, bird's eggs (Darnerud *et al.*, 2001; De Wit, 2002; Law *et al.*, 2003; Sjodin *et al.*, 1999), as well as in human blood, adipose tissue (Covaci *et al.*, 2002; Meironyte *et al.*, 1999), and breast milk samples (Noren and Meironyte, 2000).

The European Union Community banned the use of penta-BDE and octa-BDE since August 2004 (European Union, Restriction of Hazardous Substances Directive; Directive 2002/95/EC of the European Parliament and of the Council of 27 January 2003 on the restriction of the use of certain hazardous substances in electrical and electronic equipment, OJ L37, page 19, 13 February 2003), voluntarily followed by one of the major U.S. producers (Great Lakes Chemical Corporation, IN…) that ceased the production of these two PBDE mixtures by the end of 2004. The continued use of deca-BDE is still under discussion within the United States, but its use is not restricted in the European Union (Commission Decision of 13 October 2005 amending for the purposes of adapting to the technical progress the Annex to Directive 2002/95/EC of the European Parliament and of the Council on the restriction of the use of certain hazardous substances in electrical and electronic equipment, OJ L271, of 15 October 2005, page 48). The current ban on PBDEs will eventually cause these chemicals to reach a steady state concentration followed by a decline in biota and abiota, as has already been reported in human breast milk samples from Sweden (Lind *et al.*, 2003). However, due to the persistent nature of PBDEs, it could take decades before this decline can be observed in all matrices world wide with continuing exposure through older end product use and waste incineration.

Structural similarities of PBDEs with other polyhalogenated aromatic hydrocarbons that bind to the aryl hydrocarbon receptor (AhR), such as some polychlorinated biphenyls (PCBs; Fig. 1), raised concern about the possible binding of PBDEs to the AhR, leading to AhR-mediated toxic and biological effects. The AhR can be found in the cytoplasm of almost all vertebrate cells and a structurally diverse range of chemicals can bind to and/or activate AhR-dependent gene expressions, which leads to a variety of biological and toxic effects (Denison and Heath-Pagliuso, 1998;Denison *et al.*, 2002). This ligand diversity suggests that the AhR has a rather promiscuous binding pocket and raises questions as to the spectrum of chemicals which can bind to the AhR. Upon binding of a ligand to this receptor, the ligand-AhR complex migrates into the nucleus of the cell where it binds the AhR nuclear translocator protein (ARNT) (Whitlock *et al.*, 1996). The AhR and ARNT function together as a heterodimer and can bind to the xenobiotic response elements (XREs) on the DNA (Reyes *et al.*, 1992;Whitelaw *et al.*, 1993). These XREs have been identified on the 5-prime promotor regions of several AhR-inducible genes (Hankinson, 2005;Kuramoto *et al.*, 2002;Lees and Whitelaw, 1999). Binding of the ligand-AhR-ARNT complex to the XRE

stimulates transcription of adjacent genes; the molecular mechanism of induction of expression of the phase I biotransformation enzyme cytochrome P4501A1 (CYP1A1) gene has been the most studied response (Nebert and Gonzalez, 1987).

CYP1A1 enhances the detoxification of lipophilic substrates (many of which are AhR ligands) that might otherwise accumulate to toxic concentrations in a cell. Induction of CYP1A1 only occurs when necessary; upon removal of the stimulus (ligand) by metabolism, CYP1A1 gene expression is no longer upregulated. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the highest affinity ligand for the AhR, and it is also extremely slowly metabolized by CYP1A1 and other enzymes. Consequently, the long residence time of TCDD in the cell results in a sustained induction of gene expression, and it is commonly thought that most of the potent toxic and biological effects of TCDD and related chemicals are attributed to the prolonged activation of the AhR-dependent gene expression (Whitlock *et al.*, 1996). CYP1A1 is inducibly expressed in all vertebrates, although there are large differences in expression levels in different tissues and among species (Denison and Heath-Pagliuso, 1998; Denison *et al.*, 2002; Guengerich, 1993). Use of induction of CYP1A1 and CYP1A1-dependent ethoxyresorufin-O-deethylation (EROD) as a biomarker for AhR-mediated effects in the risk assessment process developed for dioxin-like compounds has been proposed and applied (Van den Berg *et al.*, 1998).

Previously, we exposed rodent hepatoma (H4IIE), human breast carcinoma (MCF-7), and human hepatoma (HepG2) cell lines to highly purified PBDEs (BDE-47, -99, -100, -153, -154, -183) (the numbering of the PBDE congeners is adapted from the International Union of Pure and Applied Chemistry system for numbering PCB congeners [Ballschmiter and Zell, 1980]) in order to assess their ability to activate the AhR-mediated CYP1A1 induction using EROD activity as a marker. While these individual PBDEs were not able to induce CYP1A1, coexposure resulted in a decrease in TCDD-induced CYP1A1 activity (Peters *et al.*, 2004). Similar results were observed for these PBDE congeners using primary hepatocytes of cynomolgus monkeys (*Macaca fascicularis*) (Peters *et al.*, 2005). In contrast to our experiments, a weak but statistically significant CYP1A1 induction has been previously reported for PBDE-77, -100, -153, and -183 in various cell lines (Behnisch *et al.*, 2003; Chen *et al.*, 2001) and primary rat hepatocytes (Chen and Bunce, 2003; Chen *et al.*, 2001).

The objectives of our present study were to assess if the observed inhibitory effects of these PBDEs were caused by direct antagonism of the AhR and/or inhibition of the CYP1A1-dependent EROD activity. In addition to measuring CYP1A1 activity, PBDE interactions with the AhR were also assessed using two stably transfected rodent hepatoma cell lines containing an AhR-responsive enhanced green fluorescent protein (AhR-EGFP) reporter gene containing approximately 500 bp of the CYP1A1 promoter including four XRE sequences (H1G1.1c3 mouse and H4G1.1c2 rat hepatoma cell lines). A human hepatoma (HepG2) cell line containing either a stably transfected construct of two isolated XRE sequences in front of a heterologous promoter (XRE-HepG2) or transiently transfected with a Gal4-AhR construct were used to investigate the AhR and XRE binding further.

MATERIALS AND METHODS

Chemicals

The chemicals used were obtained from the following companies. TCDD (>99% pure) was purchased from Cambridge Isotope Laboratories (Woburn, MA); environmentally relevant PBDE congeners (> 98% pure; Åke Bergman, Sweden) were synthesized and each congener was subjected to a specific purification on activated charcoal and Celite to remove possible contamination with dioxin-like compounds such as polybrominated dibenzofurans (Marsh *et*

al., 1999). Besides using the earlier mentioned environmentally relevant PBDEs, BDE-77 was also included in our experiments. BDE-77 is not environmentally relevant but resembles PCB-77 due to its lack of *ortho*-bromine and two adjacent bromine atoms on both aromatic rings and was therefore included. H1G1.1c3 and H4G1.1c2 cell lines were provided by M. S. Denison (University of California—Davis, CA), and XRE-HepG2 cells were provided by K. Gradin (University of Stockholm, Stockholm, Sweden); the HepG2 cell line was obtained from American Type Culture Collection (Manassa, VA). Cell culture media Dulbecco's Modified Eagle Medium (DMEM), RPMI1640 with glutamine and phenol red, phosphate buffered saline (PBS), and fetal calf serum (FCS) were obtained from Gibco BRL (Breda, The Netherlands). Fugene 6 was purchased from Roche (Mannheim, Germany), luciferase cell culture lysis buffer (5×) reagent was purchased from Promega (Madison, WI), and luciferase buffer and adenosine triphosphate (ATP) substrate were purchased from BioThema (Handen, Sweden). Polymerase chain reaction (PCR) reagents as well as OptiMEM were from Invitrogen (Carlsbad, CA), and the protease inhibitor mix was from Roche. All other chemicals were obtained from Sigma Chemical Company (St Louis, MO).

Cell culture

The mouse hepatoma cell line H1G1.1c3 and rat hepatoma cell line H4G1.1c2 were created by stable transfection of mouse hepatoma (Hepa1c1c7) and rat hepatoma (H4IIE) cells with the AhR-EGFP reporter plasmid pGreen1 as reported earlier (Nagy *et al.*, 2002). Both cell lines were cultured in DMEM, supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin in an incubator (5% CO_2, 37°C).

The HepG2-derived cell line XRE-HepG2 was created by stable transfection with a construct containing XRE sequences (Gradin *et al.*, 1993). The human hepatoma cell lines HepG2 and XRE-HepG2 were cultured in RPMI medium with phenol red and glutamine, supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. The XRE-HepG2 medium was additionally supplemented with 800 μg/ml geneticin.

Enhanced green fluorescent protein assay

The H1G1.1c3 and H4G1.1c2 cell lines have been genetically modified to produce a fluorescent protein upon activation of the AhR by ligands. Both cell lines were seeded in normal culture medium in 96-well plates (1×10^4 cells per well) and exposed after 24 h to the solvent control dimethyl sulfoxide (DMSO) (0.1%), positive control TCDD (0.001–1nM), and the chosen PBDEs (0.01–10μM) or coexposed to both TCDD and PBDEs. After 72 h, the cells were washed twice with PBS (37°C), PBS added to each well, and the fluorescence of intact cells measured using an excitation wavelength of 485 nm and emission wavelength of 510 nm in a Fluostar (BMG Labtechnologies GmbH, Offenburg, Germany). Dose response relationship analyses for the TCDD, PBDEs, and TCDD/PBDEs coexposures were carried out, and induced AhR-EGFP activity was determined by subtracting the background fluorescence in the DMSO sample from the TCDD-, PBDE-, or TCDD/PBDE-treated samples (Galietta *et al.*, 2001).

7-Ethoxyresorufin-O-deethylase assay

EROD activity was used as a marker for CYP1A1-mediated catalytic activity using a modification of the method described by Burke and Mayer (1974) as reported recently (Peters *et al.*, 2004). Directly following the AhR-EGFP assay, the PBS in the microplate wells was replaced with serum-free medium supplemented with 5mM $MgCl_2$, 5μM 7-ethoxyresorufin (ER), and 10mM dicumarol. The conversion of 7-ER into the fluorescent resorufin was followed over a 10-min period at 37°C using an excitation wavelength of 530

nm and emission wavelength of 590 nm. The EROD activity was normalized to the amount of protein per well as measured by the method of Denizot and Lang (1986).

Transient transfection and luciferase assays

The Gal4-AhR construct and the corresponding Gal4-Luc reporter gene were created by cloning of the rat AhR into the mammalian expression vector pFA-CMV containing the cDNA for the yeast Gal4 DNA-binding domain as described by Backlund and Ingelman-Sundberg (2004). Transient transfections into HepG2 cells were carried out in 24-well plates, using 150 ng of pFA-AhR expression plasmid and 200 ng of pFR-Luc per well in OptiMEM, as previously reported (Backlund and Ingelman-Sundberg, 2004). Fugene 6 transfection reagent was used according to the manufacturer's instructions (1.5 μl per well). Approximately 24 h (5% CO_2, 37°C) after transfection, the culture medium was exchanged with fresh media containing solvent control DMSO (0.1%), TCDD (0.001–1nM), BDE-99 (0.01–10μM), or a combination of TCDD and BDE-99. After 24 h (5% CO_2, 37°C), the transiently transfected cells (24-well plates) or stable XRE-HepG2 cells (12-well plates) were rinsed with PBS, and 100–60 μl lysis buffer was added (12–24 well, respectively). The cell lysates were collected by scraping and transferred to a microcentrifuge tube. After brief vortexing, cell lysates were spun (2–5 min, 4°C, maximum rpm), and 30 μl of the supernatant was mixed with 100 μl Luciferin substrate and 100 μl ATP substrate and luciferase activity determined in a luminometer. The values were normalized to the protein concentrations measured according to the method of Bradford (1976).

Chromatin immunoprecipitation assay

The procedure for the chromatin immunoprecipitation (ChIP) assay was adopted from that described by DiRenzo *et al.* (DiRenzo *et al.*, 2000). HepG2 cells were seeded in cell culture dishes (15 cm diameter) and grown until 80% confluency. The cells were then treated with DMSO (0.1%), TCDD (1nM), BDE-99 (10 μM), or TCDD and BDE-99 (1nM and 10μM, respectively). After a 3-h incubation period, cells were washed with PBS. Protein cross-linking was achieved by adding formaldehyde (1%) to the cells and allowing them to incubate at room temperature for 10 min. Cells were washed twice with ice-cold PBS, collected into a 100mM Tris-HCL (pH 8.7) and 10mM dithiothreitol solution (cold) into a centrifuge tube, incubated for 15 min at 30°C, and subsequently pelleted (5 min, 2000 g). The pellets were washed sequentially in the following buffers: PBS (ice cold), buffer I (0.25% Triton X-100, 10mM ethylenediaminetetraacetic acid [EDTA], 0.5mM ethyleneglycol-bis(2-aminoethyl ether)-N,N,N′, N-tetraacetic acid (EGTA), 10mM 4-(2-hydroxvethyl)-1-piperazineethanesulfonic acid (HEPES) [pH 6.5]), and buffer II (200mM NaCl, 1mM EDTA, 0.5mM ethyleneglycol-bis(2-aminoethyl ether)-N,N,N′, N′-tetraacetic acid (EGTA), 10mM 4-(2-hydroxvethyl)-1-piperazineethanesulfonic acid (HEPES) [pH 6.5]). The pellets were resuspended in 0.5 ml lysis buffer (1% SDS, 10mM EDTA, 50mM Tris-HCl [pH 8.0], protease inhibitor cocktail) and then sonicated on ice (three times for 10 s, Branson Sonifier) to yield DNA fragments in the 200- to 900-bp range. Samples were centrifuged for 10 min at 4°C, and the supernatants were mixed (1:10) with dilution buffer (1% Triton X-100, 2mM EDTA, 150mM NaCl, 20mM Tris-HCl [pH 8.0], protease inhibitor cocktail). IgG immunoclearing was performed by incubating 1 ml soluble chromatin with 2 μg sheared herring sperm DNA, 6 μg IgG, and 45 μl protein G-sepharose (50% slurry) for 2 h at 4°C with rotation. After centrifugation (15 s, 3000 rpm), the supernatant was incubated overnight with 5 μg of AhR-specific antibodies or IgG (overnight at 4°C with rotation). After this, 45 μl of protein G-sepharose slurry containing 2 μg sheared herring sperm DNA was added to the samples (2 h at 4°C rotation). The sepharose beads were collected by centrifugation (15 s, 3000 rpm) and washed sequentially in the following buffers (15-min rotation): TSE I (20mM Tris-HCL [pH 8.1], 150mM NaCl, 2mM EDTA, 0.1% SDS, 1% Triton X-100), TSE II (20mM Tris-HCL [pH 8.1], 500mM NaCl, 2mM EDTA, 0.1% SDS,

1% Triton X-100), buffer III (10mM Tris-HCL [pH 8.1], 0.5M LiCl, 1% nonidet P-40, 1% deoxycholate, 1mM EDTA), and twice with TE-buffer (pH 8.0). Immunocomplexes were extracted three times from the beads with 100 μl 1% SDS-0.1M $NaHCO_3$ and centrifuged at 3000 rpm for 15 s.

Cross-linking was reversed by heating the eluates at 65°C overnight. The eluates were then purified with QIAquick Spin Kit, and 1 μl of the purified DNA (0.1 μl for the input sample) was used for PCR (Platinum *Pfx* DNA Polymerase; performed according to the manufacturer's recommendations) with the primers that would amplify regions containing the XRE elements of the human *CYP1A1* gene (hCYP1A1-XRE 5′-CAC CCT TCG ACA GTT CCT CTC CCT and hCYP1A1-XRE 3′-CTC CCG GGG TGG CTA GTG CTT TGA). The PCR products were separated and visualized in a 2% agarose gel.

Cell viability

After incubation with the designated compounds at concentrations used in the experiments for 72 h (5% CO_2, 37C°), H1G1.1c3, H4G1.1c2, HepG2, or XRE-HepG2 cells were washed, and medium was replaced with a 1 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Company) solution. The conversion of MTT to formazan in the presence of the mitochondrial enzymes succinate de-hydrogenase was allowed to proceed for 30 min at 37°C (Denizot and Lang, 1986). After the incubation period, cells were washed and the formazan was extracted with 0.1 ml of isopropanol and incubated for 10 min, and the formazan concentration was determined spectrophotometrically using an absorbance wavelength of 595 nm.

Statistical analysis

In each experiment, exposure to the test compound was carried out in triplicate. EC_{50} values for EROD activity and AhR-EGFP response (50% of the maximum activity, calculated using the fitted concentration-response curve) were obtained using sigmoidal dose-response nonlinear regression curve fit (GraphPad Prism 3.0, GraphPad Software Inc., San Diego, CA). Statistical differences among treatments were determined by a two-tailed Student's *t*-test, with a significance level at >95% ($p < 0.05$).

RESULTS

Exposure of mouse H1G1.1c3 and rat H4G1.1c2 cells to the positive control agonist TCDD resulted in a concentration- and time-dependent increase in TCDD-induced CYP1A1 (EROD) activity and AhR-EGFP reporter gene expression. No difference in sensitivity in induction between the H1G1.1c3 and H4G1.1c2 cell lines was observed with the EROD assay (Fig. 2A), with EC_{50} values for TCDD of 8.18×10^{-12}M and 4.67×10^{-12}M, respectively. However, with respect to AhR-EGFP induction (Fig. 2B), the mouse hepatoma cell line was approximately twofold more sensitive, with EC_{50} values of 4.03×10^{-12}M for H1G1.1c3 cells and 9.16×10^{-12}M for H4G1.1c2 cells. These almost similar EC_{50} values were caused by the fact that the mouse H1G1.1c3 cell line reached a higher maximum induction (Fig. 2B). Although these cell lines are exquisitely sensitive bioassays to detect inducers of AhR-dependent gene expression, no induction of EGFP or CYP1A1/EROD was observed for any individual PBDE (data not shown).

Antagonistic Effects of PBDEs on AhR-EFGP Expression

Co-incubation of TCDD (0.001–1nM) with 0.1–10μM PBDEs (BDE-47, -99, -100, -153, -154, -183 and -77) resulted in a concentration-dependent decrease in AhR-EGFP expression (Fig. 3A–3C). This antagonistic effect was observed for almost all PBDEs tested, although the planar BDE-77 exhibited the greatest antagonistic effect in both cell lines (Fig.

3B). A quantitative difference in antagonistic effects was observed between the PBDEs tested, which appeared related to their degree of bromination. Lower brominated PBDEs such as the tetra-brominated BDE-47 and -77 showed stronger antagonism of TCDD-induced AhR-EGFP activity compared to higher brominated PBDEs, while the highest brominated congener tested (hepta-brominated BDE-183) failed to antagonize AhR-EGFP reporter gene induction (Fig. 3C).

No striking differences were observed between the two cell lines, though the AhR-EGFP expression resulted in more significant effects in the rat hepatoma cell line (Tables 1 and 2).

Antagonistic Effects of PBDEs on EROD Activity

Consistent with the above results, a concentration-dependent decrease in EROD activity was also observed (Fig. 4). These effects occurred at higher concentrations than measured in the AhR-EGFP assay, resulting in lower EC_{50} values (Tables 1 and 2). As with the AhR-EGFP assay, this antagonistic/inhibitory effect was more pronounced with the lower brominated PBDEs, with the planar BDE-77 being the most potent antagonist in both cell lines (Fig. 4B). No obvious differences were observed between the two cell lines, though the mouse hepatoma cell line showed a more pronounced effect in the EROD assay as can be seen in Tables 1 and 2.

Transfections in HepG2 Cells

The human hepatoma cell line (HepG2) containing a stably transfected XRE-driven luciferase reporter gene was also used to study the possible AhR-mediated dose- and time-dependent activation of transcription by different PBDEs. Exposure of the cells to the positive control TCDD resulted in a dose-dependent significant induction (EC_{50} 6.7×10^{-10}M). The PBDEs resulted in a small though not always significant induction of XRE-driven luciferase activity (at most 2% of that of TCDD 1nM for BDE-99 10μM) compared to the solvent control. The planar BDE-77 that proved the most potent antagonist in the EROD and AhR-EGFP assays did not result in a remarkably higher induction of XRE-driven luciferase activity compared to other lower brominated congeners as BDE-47 and BDE-99 (data not shown). BDE-99 resulted in the most consistent significant luciferase activity.

After co-incubation of the XRE-HepG2 cells with TCDD and selected PBDEs, the human cell line responded similarly to the rodent cell lines. All PBDEs caused an antagonistic effect toward the TCDD-induced XRE induction. The AhR-EGFP construct contains 500 bp of the CYP1A1 promotor and the HepG2-XRE construct contains isolated XRE sequences; the fact that the PBDEs are able to inhibit TCDD-induced responses in both systems is an indication that the inhibition is mediated via the XRE sequences.

Because the most consistent results in the XRE-HepG2 luciferase assay were obtained with BDE-99, HepG2 cells were transiently transfected with a Gal4-AhR construct and the corresponding Gal4-Luc reporter gene and exposed to BDE-99, with and without co-exposure to the TCDD. Exposure to TCDD resulted in a dose-dependent increase in luciferase activity in this cell line (EC_{50} 3.7×10^{-10}M). However, exposure to BDE-99 (0.01–10μM) alone did not result in a significant induction of AhR expression (Fig. 5). The Gal4-AhR expression is driven by the CMV-promotor and the activity of the co-transfected Gal4-dependent luciferase reporter gene is only detected after stimulation of the cells with AhR activators. Dimerization of the AhR-ARNT complex with the DNA is not needed to obtain a response in cells transiently transfected with this construct. Thus, BDE-99 by itself did not activate the AhR-XRE complex. Co-exposure of the cells with TCDD and BDE-99

resulted again in a dose-dependent decrease in reporter expression, suggesting a similar type of interaction as observed in the AhR-EGFP cell lines.

Chromatin Immunoprecipitation Assay

To further assess at what point BDE-99 was able to interfere with the signal transduction pathway, a ChIP assay was performed with cells treated with DMSO (0.1%), TCDD (1nM), BDE-99 (10 μM), or TCDD and BDE-99 (1nM and 10μM, respectively). The AhR antibody resulted in a positive band for both BDE-99 and the co-exposed cells, but the brightest band appeared with TCDD-exposed cells (Fig. 6). This supports the data obtained in the AhR-EGFP and transfection assays, further suggesting that some PBDEs can bind to and stimulate AhR DNA binding but that the interaction with DNA and chromatin leads to an unproductive complex incapable of activating transcription of the *CYP1A1* gene. While similar types of antagonism have been observed for some steroid hormone nuclear receptors, this would be the first AhR antagonist that exerts its inhibitory effects in this manner. The control antibody IgG did not result in any unexpected bands on the gel (Fig. 6).

DISCUSSION

PBDEs have been suggested to act as AhR antagonists based on their ability to inhibit TCDD-induced CYP1A1 activity in human and rodent cell lines (Peters *et al.*, 2004, 2005). In the present study, we determined whether these PBDEs could antagonize TCDD-induced AhR-mediated effects by direct effect on binding and/or activation of the AhR.

In both AhR-EGFP rodent cell lines, exposure to the PBDEs alone did not result in any AhR-mediated signals. As could be expected from a mechanistic point of view, this also resulted in a lack of CYP1A1 induction. However, co-exposure with TCDD resulted in a significant decrease in TCDD-induced CYP1A1 (EROD) activity in these AhR-EGFP cell lines (Fig. 3). These results are in agreement as those observed earlier with the rodent H4IIE rat hepatoma cell line, human hepatoma HepG2, and breast carcinoma MCF-7 cell lines (Peters *et al.*, 2004). For the lower brominated PBDEs, like BDE-47, this decrease in EROD activity concurred with a decrease in AhR-EGFP reporter gene expression (Figs 3A and 4A). In these instances, the antagonism in AhR-EGFP expression indicates that the antagonistic effect on TCDD-induced CYP1A1 by PBDEs is related to AhR binding and activation rather than simple competitive inhibition of EROD activity. This would also suggest that PBDEs could bind to the AhR as antagonists and compete with more potent agonists for binding the AhR in analogy with what was found for PCBs (Bandiera *et al.*, 1982;Gasiewicz *et al.*, 1996). However, some of the higher brominated PBDEs like BDE-183 also reduced TCDD-induced CYP1A1 (EROD) activity (Fig. 4C) while not influencing the AhR-EGFP gene expression (Fig. 3C). This might suggest that some of these PBDEs could reduce EROD activity by virtue of their ability to act as competitive CYP1A1 substrates as has been observed for other chemicals such as the PCBs (Bandiera *et al.*, 1982;Gasiewicz *et al.*, 1996).

In our experiments, the planar BDE-77 showed the strongest inhibitory effect on TCDD-induced CYP1A1 activity and AhR-EGFP expression. This clearly suggests that the substitution pattern of PBDE congeners that presumably bind the AhR is of importance for the observed antagonistic effects (the planarity of the congeners could facilitate binding to the AhR ligand–binding domain). In analogy with the observed effects of the bromine substitution pattern, previously Suh *et al.* (2003) reported that the chlorination substitution pattern of certain di-*ortho*–substituted PCB congeners determine their ability to elicit antagonistic effects on TCDD-induced AhR-mediated effects. Antagonistic effects by the higher chlorinated PBDEs were also observed at the XRE level. Binding to the XREs on the DNA was indirectly assessed by the use of synthetic reporter genes, which contain XRE-

binding sites in a heterologous promotor context, that reports the increased basal activity (Weiss *et al.*, 1996).

Binding to the XRE on DNA as indirectly measured in our experiments suggests that some of these PBDEs are capable of initiating the AhR transformation as well as the nuclear localization. All PBDEs tested in our study elicited an inhibitory effect on TCDD-induced XRE-driven luciferase activity. However, based on our data, it seems that the affinity of the higher brominated congeners is lower than that of the lower brominated congeners with regard to AhR-mediated effects.

A further analysis of the effects of BDE-99, the congener giving the most consistent and pronounced inhibitory effects in our present study, was performed with the Gal4-AhR construct. Singular exposure to BDE-99 did not lead to significant effects on the AhR, but the ChIP assay confirmed the ability of BDE-99 (10μM) to allow the AhR to bind to the promotor region on the DNA (Fig. 6).

It has been suggested that certain di-*ortho*–substituted PCBs bind the AhR but show no CYP1A1 induction, depending on the substitution pattern (Chen and Bunce, 2004; Merchant *et al.*, 1992; Suh *et al.*, 2003). However, this ligand binding to the AhR is probably unproductive as suggested previously for PCB-153 (Chen and Bunce, 2004; Merchant *et al.*, 1992). We suggest that, concerning the antagonistic effects of PBDEs with TCDD, there is a similar mechanism of action because ligand binding to the AhR alone is not sufficient for achieving later steps in the signaling pathway such as CYP1A1 induction, as was suggested earlier by Chen and Bunce (2004).

In summary, our results confirm the previous findings that suggest that PBDEs can bind but not activate the AhR-XRE complex and subsequent transcription processes like that of CYP1A1 (Peters *et al.*, 2004, 2005). This effect appears to be most pronounced for the lower brominated congeners, which antagonize TCDD-dependent transcriptional activation by the AhR. The mechanism for this is unknown, but as BDE-99 can cause nuclear translocation of the AhR with transcriptional activation not being initiated, this compound could cause an unfavorable conformational change of the receptor that does not allow binding to cofactors and/or components of the initiation complex. Observed interactions for some of the higher brominated PBDEs on CYP1A1 activity may be AhR independent and likely occur by competitive inhibition of CYP1A1-dependent EROD activity, but this remains to be confirmed.

Because the concentrations of both TCDD and individual PBDEs in our *in vitro* experiments far exceed the current levels in human blood or plasma, it is impossible to draw conclusions from these antagonistic interactions between both groups of compounds for human health. However, the observed effects might still have implications for the risk assessment as humans are exposed to a complex mixture with a large number of dioxin-like compounds, PBDEs and PCBs. As many quantitatively important PBDEs and PCBs can apparently act as AhR antagonists and most likely act in concert when present in mixtures, this could actually influence the overall effect of dioxin-like compounds in a downregulating way. Based on our *in vitro* results, the question remains to which extent these PBDEs could actually inhibit or antagonize AhR-mediated toxicological and biological effects *in vivo*, which should clearly be assessed further.

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References

Backlund M, Ingelman-Sundberg M. Different structural requirements of the ligand binding domain of the aryl hydrocarbon receptor for high- and low-affinity ligand binding and receptor activation. Mol Pharmacol 2004;65:416–425. [PubMed: 14742684]

Ballschmiter K, Zell M. Baseline studies of the global pollution. I. Occurrence of organohalogens in pristine European and Antarctic aquatic environments. Int J Environ Anal Chem 1980;8:15–35. [PubMed: 7399798]

Bandiera S, Safe S, Okey AB. Binding of polychlorinated biphenyls classified as either phenobarbitone-3-methylcholanthrene- or mixed-type inducers to cytosolic Ah receptor. Chem-Biol Interact 1982;39:259–277. [PubMed: 6804100]

Behnisch PA, Hosoe K, Sakai S. Brominated dioxin-like compounds: In vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. Environ Int 2003;29:861–877. [PubMed: 12850102]

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–254. [PubMed: 942051]

Burke MD, Mayer RT. Ethokyresorufin: direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methycholanthrene. Drug Metab Dispos 1974;2:583–588. [PubMed: 4155680]

Chen G, Bunce NJ. Polybrominated diphenyl ethers as Ah receptor agonists and antagonists. Toxicol Sci 2003;76:310–320. [PubMed: 14514964]

Chen G, Bunce NJ. Interaction between halogenated aromatic compounds in the Ah receptor signal transduction pathway. Environ Toxicol 2004;19:480–489. [PubMed: 15352264]

Chen G, Konstantinov AD, Chittim BG, Joyce EM, Bols NC, Bunce NJ. Synthesis of polybrominated diphenyl ethers and their capacity to induce CYP 1A1 by the Ah receptor mediated pathway. Environ Sci Technol 2001;35:3749–3756. [PubMed: 11783655]

Covaci A, de Boer J, Ryan JJ, Voorspoels S, Schepens P. Distribution of organobrominated and organochlorinated contaminants in Belgian human adipose tissue. Environ Res 2002;88:210–218. [PubMed: 12051799]

Darnerud PO, Eriksen GS, Johannesson T, Larsen PB, Viluksela M. Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. Environ Health Perspect 2001;109(Suppl 1):49–68. [PubMed: 11250805]

Denison M, Pandini A, Nagy S, Baldwin E, Bonati L. Ligand binding and activation of the Ah receptor. Chem-Biol Interact 2002;141:3. [PubMed: 12213382]

Denison MS, Heath-Pagliuso S. The Ah receptor: A regulator of the biochemical and toxicological actions of structurally diverse chemicals. Bull Environ Contam Toxicol 1998;61:557–568. [PubMed: 9841714]

Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 1986;89:271–277. [PubMed: 3486233]

De Wit CA. An overview of brominated flame retardants in the environment. Chemosphere 2002;46:583–624. [PubMed: 11999784]

DiRenzo J, Shang Y, Phelan M, Sif S, Myers M, Kingston R, Brown M. BRG-1 is recruited to estrogen-responsive promoters and cooperates with factors involved in histone acetylation. Mol Cell Biol 2000;20:7541–7549. [PubMed: 11003650]

Galietta LJ, Haggie PM, Verkman AS. Green fluorescent protein-based halide indicators with improved chloride and iodide affinities. FEBS Lett 2001;499:220–224. [PubMed: 11423120]

Gasiewicz TA, Kende AS, Rucci G, Whitney B, Willey JJ. Analysis of structural requirements for Ah receptor antagonist activity: Ellipticines, flavones, and related compounds. Biochem Pharmacol 1996;52:1787–1803. [PubMed: 8986142]

Gradin K, Wilhelmsson A, Poellinger L, Berghard A. Nonresponsiveness of normal human fibroblasts to dioxin correlates with the presence of a constitutive xenobiotic response element-binding factor. J Biol Chem 1993;268:4061–4068. [PubMed: 8382688]

Guengerich FP. Cytochrome P450 enzymes. Am Sci 1993;81:440–447.

Hankinson O. Role of coactivators in transcriptional activation by the aryl hydrocarbon receptor. Arch Biochem Biophys 2005;433:379–386. [PubMed: 15581594]

Kuramoto N, Goto E, Masamune Y, Gion K, Yoneda Y. Existence of xenobiotic response element binding in Dictyostelium. Biochim Biophys Acta 2002;1578:1–11. [PubMed: 12393182]

Law RJ, Alaee M, Allchin CR, Boon JP, Lebeuf M, Lepom P, Stern GA. Levels and trends of polybrominated diphenylethers and other brominated flame retardants in wildlife. Environ Int 2003;29:757–770. [PubMed: 12850094]

Lees MJ, Whitelaw ML. Multiple roles of ligand in transforming the dioxin receptor to an active basic helix-loop-helix/PAS transcription factor complex with the nuclear protein Arnt. Mol Cell Biol 1999;19:5811–5822. [PubMed: 10409767]

Lind Y, Darnerud PO, Atuma S, Aune M, Becker W, Bjerselius R, Cnattingius S, Glynn A. Polybrominated diphenyl ethers in breast milk from Uppsala County, Sweden. Environ Res 2003;93:186–194. [PubMed: 12963403]

Marsh G, Hu J, Jakobsson E, Rahm S, Bergman A. Synthesis and characterization of 32 polybrominated diphenyl ethers. Environ Sci Technol 1999;33:3033–3037.

Meironyte D, Noren K, Bergman A. Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972–1997. J Toxicol Environ Health A 1999;58:329–341. [PubMed: 10580757]

Merchant M, Morrison V, Santostefano M, Safe S. Mechanism of action of aryl hydrocarbon receptor antagonists: Inhibition of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced CYP1A1 gene expression. Arch Biochem Biophys 1992;298:389–394. [PubMed: 1329656]

Nagy SR, Sanborn JR, Hammock BD, Denison MS. Development of a green fluorescent protein-based cell bioassay for the rapid and inexpensive detection and characterization of ah receptor agonists. Toxicol Sci 2002;65:200–210. [PubMed: 11812924]

Nebert DW, Gonzalez FJ. P450 genes: Structure, evolution, and regulation. Annu Rev Biochem 1987;56:945–993. [PubMed: 3304150]

Noren K, Meironyte D. Certain organochlorine and organo-bromine contaminants in Swedish human milk in perspective of past 20–30 years. Chemosphere 2000;40:1111–1123. [PubMed: 10739053]

Peters AK, Sanderson JT, Bergman A, Van den Berg M. Antagonism of TCDD-induced ethoxyresorufin-o-deethylation activity by polybrominated diphenyl ethers (PBDEs) in primary cynomolgus monkey (Macaca fascicularis) hepatocytes. Toxicol Lett. 2006 (in press).

Peters AK, van Londen K, Bergman A, Bohonowych J, Denison MS, van den Berg M, Sanderson JT. Effects of polybrominated diphenyl ethers on basal and TCDD-induced ethoxyresorufin activity and cytochrome P450-1A1 expression in MCF-7, HepG2, and H4IIE cells. Toxicol Sci 2004;82:488–496. [PubMed: 15456928]

Reyes H, Reisz-Porszasz S, Hankinson O. Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. Science 1992;256:1193–1195. [PubMed: 1317062]

Sjodin A, Hagmar L, Klasson-Wehler E, Kronholm-Diab K, Jakobsson E, Bergman A. Flame retardant exposure: Polybrominated diphenyl ethers in blood from Swedish workers. Environ Health Perspect 1999;107:643–648. [PubMed: 10417362]

Suh J, Kang JS, Yang KH, Kaminski NE. Antagonism of aryl hydrocarbon receptor-dependent induction of CYP1A1 and inhibition of IgM expression by di-ortho-substituted polychlorinated biphenyls. Toxicol Appl Pharmacol 2003;187:11–21. [PubMed: 12628580]

Van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, et al. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 1998;106:775–792. [PubMed: 9831538]

Watanabe, I.; Tatsukawa, R. Workshop on Brominated Aromatic Flame Retardants. National Chemicals Inspectorate; Sweden, Skokloster, Sweden: 1989. Anthropogenic Brominated Aromatics in the Japanese Environment.

Weiss C, Kolluri SK, Kiefer F, Gottlicher M. Complementation of Ah receptor deficiency in hepatoma cells: Negative feedback regulation and cell cycle control by the Ah receptor. Exp Cell Res 1996;226:154–163. [PubMed: 8660951]

Whitelaw M, Pongratz I, Wilhelmsson A, Gustafsson JA, Poellinger L. Ligand-dependent recruitment of the Arnt coregulator determines DNA recognition by the dioxin receptor. Mol Cell Biol 1993;13:2504–2514. [PubMed: 8384309]

Whitlock JP Jr, Okino ST, Dong L, Ko HP, Clarke-Katzenberg R, Ma Q, Li H. Cytochromes P450 5: Induction of cytochrome P4501A1: A model for analyzing mammalian gene transcription. FASEB J 1996;10:809–818. [PubMed: 8666157]

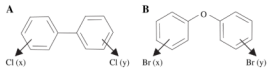

FIG. 1.
Structure of (A) PCBs and (B) PBDEs, with ($x + y \leq 10$).

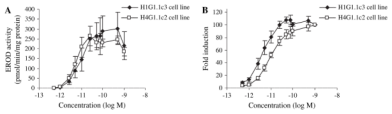

FIG. 2.

Induction of (A) EROD-activity and (B) AhR-EGFP expression in rat H4G1.1c2 and mouse H1G1.1c3 cells, after exposure to the positive control TCDD. The data are expressed as mean of three separate experiments ± SEM.

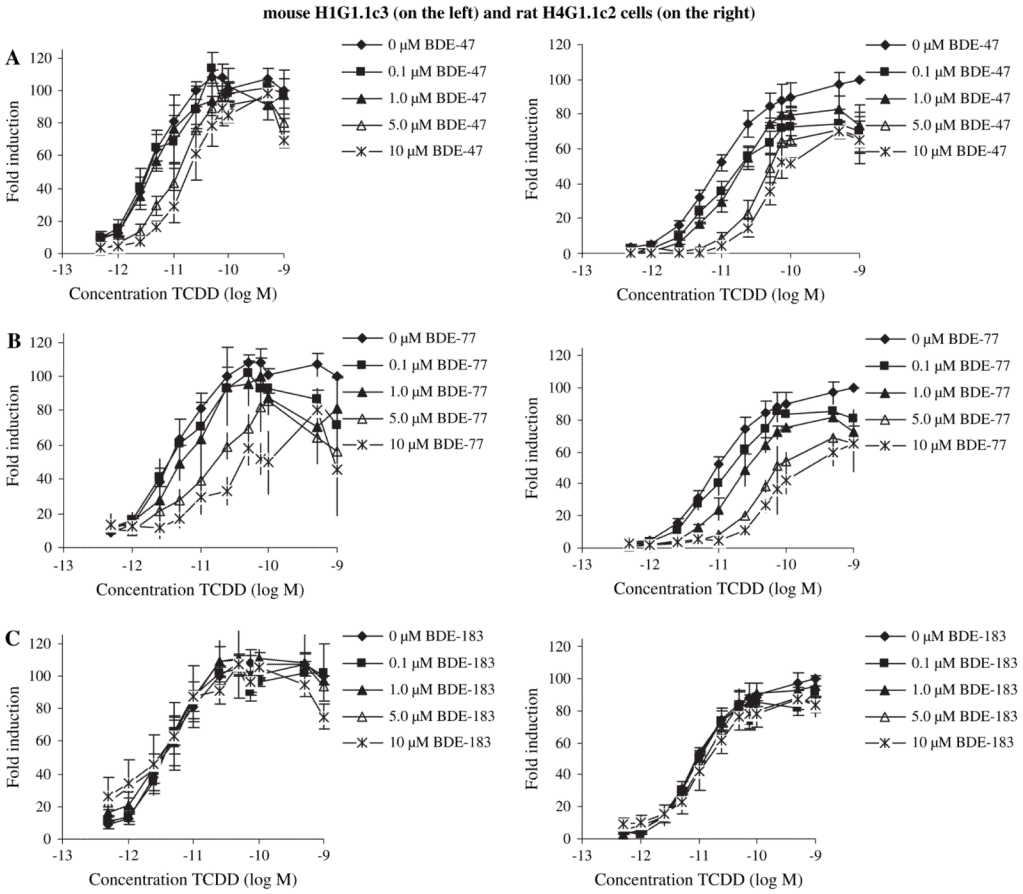

FIG. 3.

Induction of AhR-EGFP expression in mouse H1G1.1c3 (left) and rat H4G1.1c2 (right) cells after coexposure to TCDD (0.5pM–1nM) and (A) BDE-47 (0–10μM), (B) BDE-77 (0–10μM), and (C) BDE-183 (0–10μM). The data are expressed as mean of three separate experiments ± SEM.

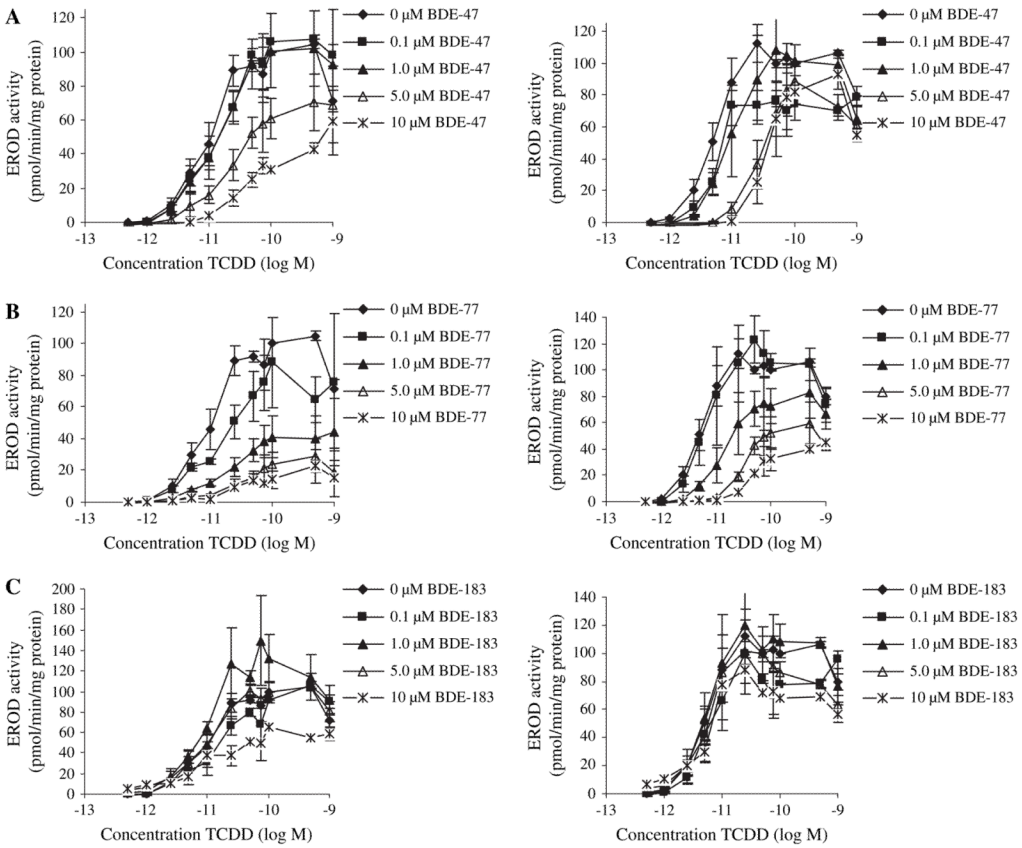

FIG. 4.

Induction of EROD activity in mouse H1G1.1c3 (left) and rat H4G1.1c2 (right) cells after coexposure to TCDD (0.5pM–1nM) and (A) BDE-47 (0–10μM), (B) BDE-77 (0–10μM), and (C) BDE-183 (0–10μM). The data are expressed as mean of three separate experiments ± SEM.

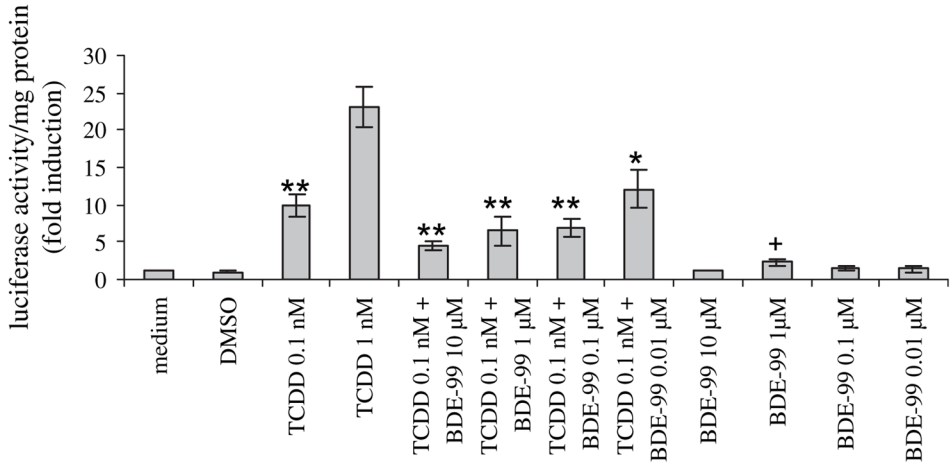

FIG. 5.

Luciferase activity in HepG2 cells transiently transfected with Gal4-AhR expression vector and the corresponding Gal4-Luc reporter gene. Cells were exposed to solvent control DMSO (0.1%), TCDD (1nM), BDE-99 (0.01–10µM), and a combination of TCDD (1nM) and BDE-99 (0.01–10µM). Data are expressed as mean of one representative assay ± SEM (n = 3, *$p < 0.05$ compared to TCDD 0.1nM): **$p < 0.01$ compared to TCDD (1nM); $^{+}$ $p < 0.05$ compared to DMSO.

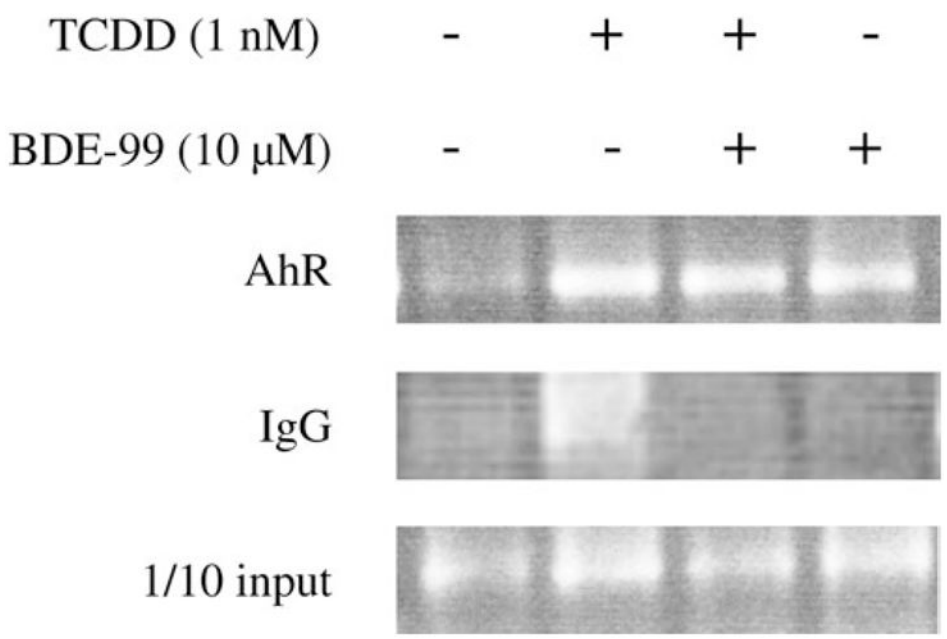

FIG. 6.
End products of the ChIP assay on a 2% agarose gel.

TABLE 1

Effect of PBDEs on TCDD-Induced AhR-EGFP Expression and EROD Activity in Mouse H1G1.c1 Cells

	Concentration (nM)	EC_{50}	EROD		Concentration (nM)	EC_{50}	AhR-EGFP	
	0.1	**8.18×10^{-12}**	**100**	***p***	**1**	**4.03×10^{-12}**	**100**	***p***
TCDD	**Concentration (μM)**		**Y-max**	***t*-Test**	**Concentration (μM)**		**Y-max**	***t*-Test**
BDE-47	0.1	1.45×10^{-11}	105.8 ± 39	0.89	0.1	3.62×10^{-12}	98.2 ± 9	0.85
	1	1.10×10^{-11}	100.9 ± 22	0.97	1	4.07×10^{-12}	97.5 ± 15	0.88
	5	1.86×10^{-11}	60.8 ± 12	0.03*	5	1.02×10^{-11}	80.3 ± 5	0.02*
	10	4.65×10^{-11}	31.1 ± 1	0.00*	10	1.57×10^{-11}	69.7 ± 5	0.00*
BDE-77	0.1	1.18×10^{-11}	88.2 ± 28	0.70	0.1	3.49×10^{-12}	71.0 ± 5	0.00*
	1	1.75×10^{-11}	40.8 ± 13	0.01*	1	5.33×10^{-12}	81.3 ± 17	0.34
	5	2.62×10^{-11}	24.1 ± 8	0.00*	5	1.01×10^{-11}	56.4 ± 27	0.18
	10	2.02×10^{-11}	14.1 ± 5	0.00*	10	2.14×10^{-11}	45.5 ± 27	0.11
BDE-99	0.1	8.89×10^{-12}	107.8 ± 31	0.81	0.1	4.33×10^{-12}	95.2 ± 25	0.85
	1	9.30×10^{-12}	96.7 ± 5	0.55	1	4.68×10^{-12}	102.2 ± 7	0.77
	5	1.49×10^{-11}	56.0 ± 11	0.02*	5	7.54×10^{-12}	88.9 ± 18	0.56
	10	2.97×10^{-11}	47.1 ± 5	0.00*	10	1.08×10^{-11}	83.8 ± 16	0.36
BDE-100	0.1	7.13×10^{-12}	130.7 ± 5	0.00*	0.1	3.39×10^{-12}	73.6 ± 4	0.00*
	1	7.91×10^{-12}	100.1 ± 10	0.99	1	3.83×10^{-12}	94.7 ± 13	0.70
	5	6.99×10^{-12}	97.4 ± 15	0.87	5	5.39×10^{-12}	74.7 ± 15	0.17
	10	8.92×10^{-12}	63.6 ± 10	0.02*	10	6.69×10^{-12}	87.2 ± 18	0.52
BDE-153	0.1	7.37×10^{-12}	116.7 ± 13	0.28	0.1	3.57×10^{-12}	90.6 ± 8	0.29
	1	7.16×10^{-12}	97.7 ± 7	0.74	1	3.80×10^{-12}	96.0 ± 7	0.61
	5	7.67×10^{-12}	66.5 ± 10	0.03*	5	5.04×10^{-12}	96.2 ± 15	0.82
	10	8.72×10^{-12}	63.3 ± 9	0.01*	10	4.90×10^{-12}	93.4 ± 9	0.52
BDE-154	0.1	7.81×10^{-12}	148.4 ± 9	0.03*	0.1	3.95×10^{-12}	93.5 ± 18	0.81
	1	6.23×10^{-12}	125.0 ± 20	0.28	1	3.43×10^{-12}	80.4 ± 3	0.00*

Toxicol Sci. Author manuscript; available in PMC 2011 February 2.

	Concentration (nM)	EC_{50}	EROD		Concentration (nM)	EC_{50}	AhR-EGFP	
	0.1	**8.18×10^{-12}**	**100**	***p***	**1**	**4.03×10^{-12}**	**100**	***p***
TCDD	**Concentration (μM)**		**Y-max**	***t*-Test**	**Concentration (μM)**		**Y-max**	***t*-Test**
	5	7.82×10^{-12}	101.9 ± 20	0.93	5	5.00×10^{-12}	76.8 ± 11	0.22
	10	5.21×10^{-12}	50.1 ± 4	0.00*	10	7.38×10^{-12}	88.1 ± 6	0.11
BDE-183	0.1	1.16×10^{-11}	91.4 ± 33	0.81	0.1	3.91×10^{-12}	102.0 ± 4	0.62
	1	7.77×10^{-12}	132.2 ± 23	0.23	1	4.73×10^{-12}	97.2 ± 14	0.85
	5	8.31×10^{-12}	98.8 ± 10	0.91	5	5.00×10^{-12}	93.7 ± 14	0.29
	10	9.97×10^{-12}	64.8 ± 35	0.37	10	4.40×10^{-12}	74.9 ± 7	0.03*

Note. Effects of PBDEs on TCDD-induced AhR-EGFP expression and EROD activity in a stably transfected mouse hepatoma cell line (H1G1.1c3). The data are presented as the mean of three experiments (± SEM), all conducted in triplicate. Y-max: maximum achieved response of the compound compared to the maximal response of TCDD.

*Statistically significant compared to the maximum induction by TCDD ($p < 0.05$).

TABLE 2

Effects of PBDEs on TCDD-Induced AhR-EGFP Expression and EROD Activity in Rat H4G1.c1 Cells

	Concentration (nM)	EC_{50}	EROD		Concentration (nM)	EC_{50}	AhR-EGFP	
	0.1	**4.67×10^{-12}**	**100**	***p***	**1**	**9.16×10^{-12}**	**100**	***p***
TCDD	**Concentration (μM)**		**Y-max**	***t*-Test**	**Concentration (μM)**		**Y-max**	***t*-Test**
BDE-47	0.1	5.45×10^{-12}	74.1 ± 8	0.03*	0.1	1.01×10^{-11}	70.0 ± 11	0.06
	1	7.95×10^{-12}	101.7 ± 10	0.87	1	1.38×10^{-11}	73.2 ± 12	0.09
	5	2.51×10^{-11}	88.7 ± 9	0.28	5	3.41×10^{-11}	66.5 ± 9	0.02*
	10	2.96×10^{-11}	82.2 ± 18	0.38	10	4.69×10^{-11}	65.2 ± 14	0.06
BDE-77	0.1	5.43×10^{-12}	105.5 ± 7	0.45	0.1	1.05×10^{-11}	80.8 ± 5	0.02*
	1	1.23×10^{-11}	72.7 ± 13	0.11	1	1.76×10^{-11}	72.5 ± 4	0.00*
	5	3.00×10^{-11}	52.3 ± 12	0.02*	5	4.38×10^{-11}	65.6 ± 15	0.09
	10	4.84×10^{-11}	32.6 ± 9	0.00*	10	6.75×10^{-11}	65.0 ± 17	0.12
BDE-99	0.1	5.60×10^{-12}	72.7 ± 1	0.00*	0.1	8.96×10^{-12}	87.5 ± 14	0.41
	1	6.66×10^{-12}	101.7 ± 15	0.91	1	9.97×10^{-12}	89.0 ± 11	0.39
	5	1.03×10^{-11}	92.1 ± 16	0.65	5	1.71×10^{-11}	80.0 ± 3	0.00*
	10	1.49×10^{-11}	115.7 ± 36	0.68	10	2.41×10^{-11}	77.8 ± 13	0.18
BDE-100	0.1	5.09×10^{-12}	173.8 ± 64	0.32	0.1	7.58×10^{-12}	89.8 ± 10	0.37
	1	5.25×10^{-12}	140.5 ± 44	0.41	1	7.55×10^{-12}	82.7 ± 7	0.08
	5	6.85×10^{-12}	127.9 ± 37	0.49	5	9.95×10^{-12}	81.0 ± 9	0.11
	10	1.00×10^{-11}	88.2 ± 18	0.56	10	1.21×10^{-11}	60.2 ± 4	0.00*
BDE-153	0.1	5.56×10^{-12}	79.7 ± 2	0.00*	0.1	7.57×10^{-12}	79.8 ± 11	0.14
	1	4.60×10^{-12}	108.3 ± 9	0.41	1	7.19×10^{-12}	78.4 ± 15	0.22
	5	3.83×10^{-12}	94.9 ± 5	0.39	5	7.01×10^{-12}	71.5 ± 5	0.01*
	10	3.63×10^{-12}	90.0 ± 13	0.48	10	7.89×10^{-12}	77.3 ± 9	0.07
BDE-154	0.1	4.53×10^{-12}	129.8 ± 15	0.11	0.1	7.46×10^{-12}	82.0 ± 4	0.01*
	1	3.96×10^{-12}	115.1 ± 12	0.29	1	7.58×10^{-12}	83.1 ± 6	0.06

	Concentration (nM)	EC_{50}	EROD		Concentration (nM)	EC_{50}	AhR-EGFP	
	0.1	**4.67×10^{-12}**	**100**	***p***	**1**	**9.16×10^{-12}**	**100**	***p***
TCDD	**Concentration (μM)**		**Y-max**	***t*-Test**	**Concentration (μM)**		**Y-max**	***t*-Test**
	5	4.11×10^{-12}	89.9 ± 12	0.45	5	7.37×10^{-12}	75.5 ± 9	0.05*
	10	4.08×10^{-12}	64.1 ± 13	0.05*	10	1.02×10^{-11}	67.8 ± 13	0.07
BDE-183	0.1	4.87×10^{-12}	77.2 ± 12	0.14	0.1	7.86×10^{-12}	92.1 ± 2	0.02*
	1	4.71×10^{-12}	108.8 ± 12	0.50	1	8.46×10^{-12}	95.9 ± 8	0.62
	5	5.15×10^{-12}	86.6 ± 7	0.15	5	1.03×10^{-11}	84.6 ± 8	0.13
	10	5.99×10^{-12}	68.2 ± 26	0.28	10	1.26×10^{-11}	83.6 ± 5	0.03*

Note. Effects of PBDEs on TCDD-induced AhR-EGFP expression and EROD activity in a stably transfected rat hepatoma cell line (H4G1.1c2). The data are presented as the mean of three experiments (± SEM), all conducted in triplicate. Y-max: maximum achieved response of the compound compared to the maximal response of TCDD.

*Statistically significant compared to the maximum induction by TCDD ($p < 0.05$).