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Effects of fifteen PBDE metabolites, DE71, DE79 and TBBPA on steroidogenesis in the H295R cell line

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Abstract

Polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBPA) are brominated flame retardants that are produced in large quantities and are commonly used in construction materials, textiles, and as polymers in electronic equipment. Environmental and human levels of PBDEs have been increasing in the past 30 years, but the toxicity of PBDEs is not fully understood. Studies on their effects are relatively limited, and show that PBDEs are neurotoxins and potential endocrine disrupters. Hydroxylated (OH—) and methoxylated (MeO—) PBDEs have also been reported in the adipose tissue, blood and milk of wild animals and humans. In the present study, 15 PBDE metabolites, two BDE mixtures (DE71 and DE79), and TBBPA were studied individually to determine their effects on ten steroidogenic genes, aromatase activity, and concentrations of two steroid hormones (testosterone and 17β-estradiol) in the H295R human adrenocortical carcinoma cell line. Exposure to 0.05 μM 2′-OH-BDE-68 significantly induced the expression of CYP11A, CYP11B2, CYP17, CYP21, 3βHSD2, 17βHSD1, and 17βHSD4, and the expression of StAR was induced by 6-OH-BDE-90 at the three exposure concentrations. Exposure to DE71 and DE79 resulted in dose-dependent trend towards induction, but these effects were not significant. Exposure to 0.5 μM 2-OH-BDE-123 and 2-MeO-BDE-123 resulted in significantly greater aromatase activity. However, none of the compounds affected sex hormone production at the concentrations tested. Generally, OH-BDEs had a much stronger ability to affect steroidogenic gene expression than MeO-BDEs.

Keywords: PBDEs; TBBPA; Steroidogenesis; H295R cells

1. Introduction

Polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBPA) are commonly used as brominated flame retardants (BFRs) in construction materials, textiles, and as polymers in electronic equipment (Brown et al., 2004). These compounds are hydrophobic, stable and persist in the environment and have the potential to bioaccumulate (Hites, 2004; Birchmeier et al., 2005). PBDEs have been found in the environment, wildlife, as well as in human blood, tissues, hair and breast-milk (de Wit, 2002), and TBBPA has been found in surface waters, wastewater and sediments (Blanco et al., 2005; Verslycke

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et al., 2005). The most predominant PBDE congeners measured in humans are BDE 47, followed by BDEs 28, 99, and 100. DE71, a pentabrominated mixture, and DE79, an octabrominated mixture, are also commonly found in biological samples. Less brominated PBDEs, such as tetra-, penta- and hexa-BDEs, demonstrate high affinity for lipids and can bioaccumulate in the tissues of wildlife and humans (Zhou et al., 2001). The use of DE71 and DE79 has been banned in Europe and in several states in the US, but there are no restrictions on the use of the deca-BDE mixture and TBBPA (EU, 2003; BSEF, 2006).

The presence of methoxylated (MeO—) and hydroxylated (OH—) PBDEs in blood, adipose tissues, and liver of fish, birds, and mammals has been reported in a few studies (Marsh et al., 2004; Sinkkonen et al., 2004; Teuten et al., 2005). Current understanding indicates that MeO-BDEs found in wildlife are the consequence of accumulation via natural sources in the marine environment (Marsh et al., 2004; Teuten et al., 2005), whereas OH-BDEs can have a natural origin and/or result from metabolism of PBDEs (Örn and Klasson-Wehler, 1998; Letcher et al., 2000; Hakk and Letcher, 2003).

The effects of PBDE and TBBPA exposure have been assessed in several systems. Some studies have suggested that tetra- and penta-BDEs were more toxic and bioaccumulative compounds compared with octa- and deca-congeners (Siddiqi et al., 2003). PBDEs have been shown to bind to the AhR as measured using ethoxyresorufin-O-deethylase (EROD) activity (Meerts et al., 2001; Mundy et al., 2004), and some PBDEs were found to activate the AhR signal transduction pathway at moderate to high concentrations (Brown et al., 2004). Exposure to PBDEs induced thyroid hyperplasia and altered thyroid hormone production and transport *in vitro* (Marsh et al., 1998; Meerts et al., 2000, 2001; Siddiqi et al., 2003). As the structure of PBDEs is similar to those of PCBs, PBDEs and their metabolites may act as endocrine disrupters by interfering with thyroid hormone homeostasis (Hooper and McDonald, 2000; Zhou et al., 2001). Previous studies suggested that liver, thyroid gland, pancreas, and kidney could be the endpoints for identifying endocrine disrupters of cytochrome P450 isozymes (Hallgren et al., 2001; Darnerud et al., 2005). Some PBDEs and their derivatives (including OH-BDEs and MeO-BDEs) were reported to be able to induce or inhibit aromatase (CYP19) and CYP17 activity in H295R cells (Cantón et al., 2005, 2006). TBBPA is also considered a potential endocrine disruptor (Kitamura et al., 2002, 2005). An *in vitro* study of BFRs showed that TBBPA binding to TTR was ten times more effective than that of T4 to TTR (Meerts et al., 2000). However, information about the endocrine activity of PBDE metabolites or derivatives is still limited.

Despite the widespread occurrence of PBDEs and TBBPA in the environment, limited information is available on their effects on steroidogenesis (Zhou et al., 2002; Darnerud et al., 2005). The H295R human adrenocortical carcinoma cell line has been characterized in detail and shown to express all key enzymes necessary for steroidogenesis (Gazdar et al., 1990). This system has been used in mechanistic studies and to provide relevant data for risk assessment based on measuring effects on specific enzymes and hormone production (Hecker et al., 2006, 2007). Therefore, this cell line can be used as a model for *in vitro* screening for both adrenocortical toxicity and steroidogenesis. In the present study, the expression of 10 key steroidogenic genes was measured (CYP11A, CYP11B, CYP17, CYP19, CYP21, 3βHSD2, 17βHSD1, 17βHSD4, HMGR and StAR) using quantitative real-time PCR (Q-RT-PCR). This technique offers a more specific, sensitive, comprehensive, and reliable method for the investigation of multiple functionally related genes (Sanderson et al., 2002; Hilscherova et al., 2004; Zhang et al., 2005).

To date, there is little information on the effects of PBDEs and their derivatives relevant to human exposure levels (1–462 ng PBDEs/g in adipose tissue) (Gill et al., 2004). The purpose of this study is to determine the effects of 15 PBDE metabolites, two mixtures, and TBBPA on steroidogenesis using the H295R cell line at relevant human exposure levels.

2. Materials and methods

2.1. Test chemicals

Of the chemicals tested, the two commercial mixtures (DE71 and DE79) and TBBPA were purchased from Sigma Chemical Co. (St. Louis, MO), while the PBDE metabolites were synthesized in the Department of Biology and Chemistry of City University of Hong Kong according to the methods described in Marsh et al. (2003) with purities >98%. Each compound was dissolved in dimethyl sulfoxide (DMSO), and the final DMSO concentration in the exposure medium was 0.5% (v/v).

2.2. Cell culture

H295R cells were obtained from the American type culture collection (ATCC CRL-2128; ATCC, Manassas, VA) and cultured in 75 cm^2 flasks at 37 °C in a 5% CO_2 atmosphere. The culture medium was a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 Nutrient mixture (Sigma Chemical Co., St Louis, MO), supplemented with 1% ITS + Premix (BD Biosciences, San Jose, CA), 2.5% Nu-Serum (BD Biosciences), 0.5% antibiotics (5000 μg/ml penicillin and 5000 μg/ml streptomycin), and 1.2 g/l Na_2CO_3. The medium was changed twice a week, and cells were detached from flasks for subculturing using trypsin/EDTA (Life Technologies Inc., Grand Island, NY).

2.3. Cell exposures

In order to measure the effects of BFR exposure on steroidogenic gene expression, H295R cells were plated onto

6-well culture plates at an initial cell density of 1×10^6 cells/ml in 2 ml cell suspension per well. After 24 h, cells were exposed to chemicals at concentrations of 0.025, 0.05 and 0.5 μM for another 48 h and then total RNA was isolated from the cells. For the aromatase and hormone measurements, cells were plated in 24-well culture plates at a density of 3×10^5 cells/ml in 1 ml per well. After 24 h, the medium was replaced and cells were exposed to chemicals at the concentrations listed above for another 48 h, after which the culture medium was collected and frozen at −80 °C for hormone measurement. The cells in the plate were gently washed twice with PBS before being used in the aromatase assay.

2.4. Cell viability assay

H295R cells were seeded into 96-well view plates at a concentration of 1×10^6 cells/ml in 200 μl of medium per well. After 24 h, cells were dosed with the PBDE metabolites. Cell viability was measured using the sulforhodamine B (SRB) assay after 48 h of exposure (Körner et al., 1998). Absorbance was subsequently measured using a plate-reading measurement system (Molecular Devices Spectra Max 340 PC).

2.5. Real-time PCR assay

The expression levels of 10 steroidogenic genes plus one housekeeping gene (β-actin) were measured following Hilscherova et al. (2004). Total RNA was isolated from exposed cells using the SV total RNA isolation system (Promega, San Luis, CA). Two microgram of cellular total RNA for each sample was used for reverse transcription using the SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). The ABI 7500 fast real-time PCR System (Applied Biosystems, Foster City, CA) was used to perform quantitative real-time PCR. PCR reaction mixtures (20 μl) contained 1 μl (0.2–0.4 μM) of forward and reverse primers, 5 μl of cDNA sample, and 10 μl of 2× SYBR Green™ PCR Master Mix (Applied Biosystems). The thermal cycle profile was denatured at 95 °C for 10 min, followed by 40 cycles of denaturation for 15 s at 95 °C, annealing together with extension for 1 min at 60 °C; and a final cycle of 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. Melting curve analyses were performed during the 60 °C stage of the final cycle to differentiate between desired PCR products and primer–dimers or DNA contaminants.

To quantify the RT-PCR results, the cycle at which the fluorescence signal was first significantly different from background (Ct) was determined for each reaction. The expression level of a target gene was normalized with reference to the β-actin endogenous control gene to derive the mean normalized expression (MNE) value (Eq. (1))

$$\mathrm{MNE} = \frac{(E_{\mathrm{reference}})C_{\mathrm{T}}^{\mathrm{reference,\ mean}}}{(E_{\mathrm{target}})C_{\mathrm{T}}^{\mathrm{target,\ mean}}}, \quad (1)$$

where $E_{\mathrm{reference}}$ and E_{target} represent the PCR efficiencies ($=10^{-1/\mathrm{slope}}$) determined from the slopes of the standard curves constructed using gene-specific RNA standards of known copy number (Simon, 2003). Gene expression levels were measured in triplicate for control and exposed cells. Levels of expression relative to solvent control were calculated (Eq. (2))

$$\text{N-fold change} = \mathrm{MNE}_{\mathrm{exp}}/\mathrm{MNE}_{\mathrm{con}}. \quad (2)$$

2.6. Aromatase activity assay

The activity of CYP19 (aromatase) was determined based on the method described by Lephart and Simpson (1991) with modifications. After medium removal and washing, the cells were exposed to 54 nM [1β-^{3}H] androstenedione (PerkinElmer, Boston, MA) in serum-free medium for 90 min at 37 °C and 5% CO_2 (Sanderson et al., 2002). Two hundred microlitre of the medium was extracted and used for measuring the level of radioactivity. Corrections were made for background radioactivity, dilution factor, and specific activity of the substrate. 8-Bromo-cyclic-adenosine-monophosphate (8-Br-cAMP, 100 μM) was used as a positive control/aromatase inducer, while 4-hydroxyandrostenedione (4-HA, 10 μM) was used as a negative control/aromatase inhibitor, as described previously (Heneweer et al., 2004).

2.7. Hormone measurement

Testosterone (T) and 17β-estradiol (E2) concentrations were measured using methods described by Hecker et al. (2006). Frozen medium was thawed on ice, and 500 μl medium was extracted twice with 2.5 ml diethyl ether. All samples were spiked with 10 μl 1,2,6,7-^{3}H-labeled T (0.0002 μCi/μl) (PerkinElmer) prior to extraction to determine extraction recoveries. The ether phase containing the target hormones was evaporated under nitrogen, and the dried extract was reconstituted in 250 μl ELISA assay buffer and frozen at −80 °C for subsequent analysis. The extracts were diluted 1:50 and 1:5 for T and E2 analysis, respectively, and the hormones levels were measured by competitive ELISA following manufacturer protocols (Cayman Chemical Company, Ann Arbor, MI).

2.8. Statistical analyses

All experiments were conducted in duplicate, and triplicate measurements were used for each individual exposure. Statistical analyses were conducted using SPSS 13 (SPSS Inc., Chicago, IL, USA). As the results were found to be reproducible and consistent between exposures, data from one representative exposure are presented as means ± standard deviations. Differences in gene expression, aromatase activity, and hormone production between control and exposed cells were evaluated by ANOVA with post-hoc

Dunnett's tests. Differences with $p < 0.05$ were considered significant.

3. Results

3.1. Cytotoxicity

None of the tested compounds were found to be cytotoxic at the exposure concentrations tested.

3.2. Effects of PBDEs and TBBPA on the expression of CYP family genes

Fifteen PBDE metabolites, two BDE mixtures (DE71 and DE79), and TBBPA were examined individually at three concentrations to study their effects on ten steroidogenic genes in H295R cell line. Five chemicals had no significant effect on any of these genes at all three concentrations (Table 1). CYP11A expression was only up-regulated by exposure to 0.05 μM 2′-OH-BDE-68 (1.7-fold). Exposure to 6-MeO-BDE-47 (0.025 μM), 5-Cl-6-OH-BDE-47 (0.5 μM), 5-Cl-6-MeO-BDE-47 (0.025 μM), and 2′-OH-BDE-68 (0.05 μM) significantly up-regulated CYP11B2 expression by 1.4-, 1.4-, 1.4-, and 2.1-fold, respectively. In contrast, exposure to 6-OH-BDE-47 (0.025, 0.05, and 0.5 μM) and 2-OH-BDE-123 (0.05 and 0.5 μM) significantly down-regulated CYP11B2 expression. The expression of CYP17 was up-regulated 1.7-fold by exposure to 0.05 μM 2′-OH-BDE-68, whereas its expression was down-regulated by exposure to 5-Cl-6-OH-BDE-47 (0.025 μM), 5-Cl-6-MeO-BDE-47 (0.5 μM), and 4′-OH-BDE-49 (0.05 and 0.5 μM). Exposure to 0.05 μM 6-OH-BDE-47 resulted in up-regulation of CYP19 expression by 1.4-fold. Moreover, CYP21 expression was also up-regulated by exposure to 6-OH-BDE-47, 2′-OH-BDE-68, 6′-Cl-2′-OH-BDE-68, 2-MeO-BDE-123, DE71, DE79, and TBBPA at various concentrations.

3.3. Effects on hydroxysteroid dehydrogenase gene expression

Among the 18 chemicals tested, only 2′-OH-BDE-68 affected hydroxysteroid dehydrogenase gene expression. Exposure to 0.05 μM 2′-OH-BDE-68 resulted in significant up-regulation of 3βHSD2, 17βHSD1, and 17βHSD4 expression by 2.1-, 2.0-, and 1.8-fold, respectively (Table 1).

3.4. Effects on StAR and HMGR gene expression

Exposure to 2′-OH-BDE-68 up-regulated the expression of StAR by 1.7-fold, while 6-OH-BDE-47 down-regulated its gene expression by about 0.3-fold. Moreover, the expression of StAR was up-regulated (2.2-fold) by exposure to 6-OH-BDE-90 at all three concentrations tested. In contrast, HMGR expression did not change in any of the treatments (Table 1).

Table 1
Effects of BFR compounds on gene expression

No.	Chemicals	Genes affected	Magnitude of effect (↑/↓ fold-change)	Effective concentration (μM)
OH-BDEs				
1	2′-OH-BDE-25	None	N.e.	N/A
2	2′-OH-BDE-28	None	N.e.	N/A
3	6-OH-BDE-47	CYP11B2	↓0.3	0.5, 0.05, 0.025
		CYP21	↑1.5	0.5
		CYP19	↑1.4	0.05
		StAR	↓0.3	0.025
4	5-Cl-6-OH-BDE-47	CYP17	↓0.4	0.025
		CYP11B2	↑1.4	0.5
5	4′-OH-BDE-49	CYP17	↓0.4	0.5, 0.05
6	2′-OH-BDE-68	CYP11A	↑1.7	0.05
		CYP11B2	↑2.1	0.05
		CYP17	↑1.7	0.05
		CYP21	↑1.9	0.05, 0.025
		3βHSD2	↑2.1	0.05
		17βHSD1	↑2.0	0.05
		17βHSD4	↑1.8	0.05
		StAR	↑1.7	0.05
7	6′-Cl-2′-OH-BDE-68	CYP21	↑1.6	0.05
8	6-OH-BDE-85	None	N.e.	N/A
9	6-OH-BDE-90	StAR	↑2.2	0.5, 0.05, 0.025
10	2-OH-BDE-123	CYP11B2	↓0.3	0.5, 0.05
MeO-BDEs				
11	6-MeO-BDE-47	CYP21	↑1.4	0.5
		CYP11B2	↑1.4	0.025
12	5-Cl-6-MeO-BDE-47	CYP11B2	↑1.4	0.5
		CYP17	↓0.4	0.5
13	4′-MeO-BDE-49	None	N.e.	N/A
14	2′-MeO-BDE-68	None	N.e.	N/A
15	2-MeO-BDE-123	CYP21	↑1.5	0.5
BDE mixtures				
16	DE71	CYP21	↑1.6	0.5
17	DE79	CYP21	↑1.6	0.5, 0.05
Other BFR				
18	TBBPA	CYP21	↑1.4	0.5

Symbols indicate fold-differences relative to control. ↑ = increased; ↓ = decreased; N/A = not applicable; and N.e. = No effect.

3.5. Effects on aromatase activity and sex hormone production

Most of the chemicals tested in this study did not significantly affect aromatase activity. Exposure to 0.05 μM DE71 resulted in significantly higher aromatase activity (mean activity was 252% of solvent control), and exposure to 0.5 μM DE71 caused a trend towards induction (393% of solvent control), although the response to this chemical was variable (Fig. 1). Exposure to the octa-BDE mixture DE79 also caused a trend towards induction of aromatase activity. Moreover, exposure to 0.5 μM 2-OH-BDE-123 and 2-MeO-BDE-123 resulted in slightly but significantly greater aromatase activity (Fig. 1). However, none of the tested compounds significantly affected sex hormone production at the exposure concentrations tested.

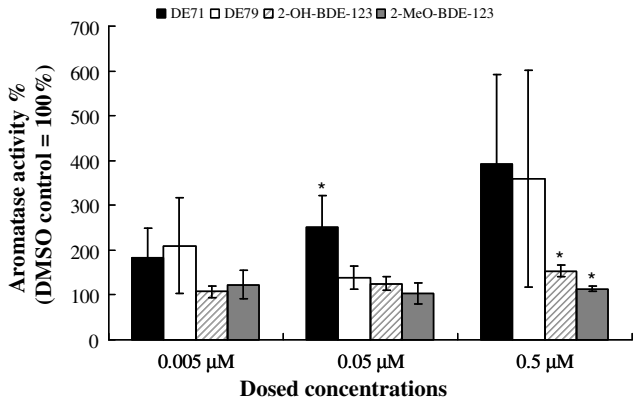

Fig. 1. Effects of exposure to DE71, DE79, 2-OH-BDE-123, and 2-MeO-BDE-123 on aromatase activity. The induction level represents the aromatase activity with respect to the 0.5% DMSO solvent control. Values presented are the means of triplicate measurements, and asterisks indicate activities that are statistically different from the DMSO solvent control ($p < 0.05$).

4. Discussion

PBDEs are widely used, and both the parent compounds and their metabolites have been shown to have endocrine disrupting effects in several test systems (e.g. Meerts et al., 2001; Cantón et al., 2005; Harju et al., 2007). However, their toxicity, and especially their effects on steroidogenesis, are not fully understood to this point. In the present study, the expression of ten key steroidogenic genes was measured by quantitative RT-PCR to observe the effects of the selected PBDEs, PBDE mixtures, and TBBPA on the H295R cell line. Thirteen of the 18 chemicals tested, especially the OH-BDE compounds, affected the expression of some genes; this observation was similar to those of previous studies in which CYP19 enzyme activity was measured (Cantón et al., 2005, 2008), and a study of the effects of other PBDE metabolites at relatively high exposure concentrations (He et al., 2008).

CYP11A catalyzes the side-chain cleavage of cholesterol, which is the starting point of steroid synthesis and it is also the rate-limiting step. Therefore, only a small change in the expression of this gene may have large effects on steroidogenesis. However, in the present study only 2′-OH-BDE-68 caused a significant up-regulation of CYP11A expression at a concentration of 0.05 μM.

CYP11B2 catalyzes the biosynthesis of the glucocorticoid cortisol from 11-deoxycortisol, which has numerous metabolic, developmental, immunosuppressive, anti-inflammatory and other functions in the body (Hilscherova et al., 2004). In the present study, four OH-BDEs (6-OH-BDE-47; 5-Cl-6-OH-BDE-47; 2′-OH-BDE-68; 2-OH-BDE-123) and two MeO-BDEs (6-MeO-BDE-47; 5-Cl-6-MeO-BDE-47) significantly affected CYP11B2, demonstrating their potential to interfere with the glucocorticoid synthesis pathway *in vitro* (Table 1). Exposure to 2′-OH-BDE-68 had the strongest effect on CYP11B2 expression, possibly because its —OH group is adjacent to a bromine atom (Cantón et al., 2005). This metabolite has been found in several species, including fish, seals, algae, and seabirds (Olsson et al., 2000; Marsh et al., 2004), and thus its potential effects on endocrine function should be investigated further *in vivo*.

CYP17 catalyzes the conversion of aldosterone to corticosteroid substrates and ultimately to sex steroid substrates, which is the initial step of cortisol biosynthesis. Therefore, it is possible that this enzyme can redirect steroid output from mineralocorticoids to glucocorticoids or weak androgens, whereas inhibition of CYP17 would have the opposite effect. A recent study reported that some OH— or MeO— PBDEs significantly inhibited CYP17 activity in H295R cells (Cantón et al., 2006). In the current study, the fact that exposure to 5-Cl-6-OH-BDE-47 resulted in down-regulation of CYP17 and up-regulation of CYP11B2 expression by 1.4-fold might be evidence of this shift in the steroidogenic pathway.

CYP19 is responsible for the final conversion of androgens to estrogens. Only 0.05 μM 6-OH-BDE-47 had an effect on CYP19 expression, and most of the chemicals tested in this study did not affect aromatase activity at any of the three dosed concentrations. Four chemicals, DE71, DE79, 2-OH-BDE-123 and 2-MeO-BDE-123, showed some ability to induce aromatase activity, although the induction levels observed were limited and variable. This result is not unexpected in the context of previous studies, as the dosed concentrations used in the current study were relatively low. A previous study reported that exposure of H295R cells to 6-OH-BDE-47 reduced aromatase (CYP19) activity at concentrations greater than 2.5 μM (Cantón et al., 2005), and another recent study reported that exposure of the same cell line to 10 μM 5-Cl-6-OH-BDE-47 significantly decreased aromatase activity and E2 production (He et al., 2008). Therefore, the environmentally relevant concentrations tested in the current study are likely too low to cause effects on aromatase activity in the H295R cell line.

Exposure to three OH-BDEs, two MeO-BDEs, two BDE mixtures and TBBPA were observed to induce CYP21 expression at different concentrations in the present study (Table 1). The CYP21 gene is required for the synthesis of both aldosterone and corticosteroids. Induction of CYP21 may lead to an increase in the synthesis of cortisol and aldosterone, and may result in a decrease in the availability of substrates for androgen and estrogen production. In the present study, CYP21 was the only gene affected by TBBPA. The lack of effects caused by TBBPA is most likely due to its short biological half life (WHO/ICPS, 1995). This substrate effect may be reflected in the aromatase induction that was observed in cells exposed to DE71, DE79 and 2′-MeO-BDE-123; more of the aromatase enzyme may be produced by the cells as a way to compensate for a reduction in the amount of androgens available for aromatization, although no change in CYP19 expression was observed after exposure to these compounds.

HSD enzymes are members of the short-chain alcohol dehydrogenase (SCAD) enzyme family. HSD enzymes

mainly catalyze two reactions: (1) the oxidation of a secondary alcohol to a ketone; and (2) the reduction of a ketone to a secondary alcohol. 3βHSD oxidizes a 3β-OH group to a C3 ketone that is an obligate step in the biosynthesis of androgens and estrogens as well as mineralocorticoids and glucocorticoids. 17β-Hydroxysteroid dehydrogenases (17HSDs) are a group of enzymes responsible for the interconversion between low-activity 17-ketosteroids and high-activity 17β-hydroxysteroids. They act as key enzymes modulating the biosynthesis and metabolism of both estrogens and androgens. In the present study, 2′-OH-BDE-68 significantly increased the expression of 3βHSD2, 17βHSD1 and 17βHSD4 by 1.8- to 2-fold, but no clear dose–response relationship was observed.

The protein encoded by the StAR gene plays a key role in the acute regulation of steroid hormone synthesis. It enhances the conversion of cholesterol to pregnenolone by mediating the transport of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane. Exposure to 2′-OH-BDE-90 caused 2.2-fold induction of StAR expression at all three concentrations, and exposure to 2′-OH-BDE-68 also up-regulated StAR by 1.7-fold. However, exposure to 6-OH-BDE-47 reduced StAR expression by about 0.3-fold. MeO-BDEs showed no effect on StAR expression.

These effects on gene expression support the idea that functional group position,, especially —OH group position, might play an important role in the effects of PBDE metabolites on steroidogenesis; other studies have also reported that hydroxylated PBDEs have stronger biological effects than either the parent compound or metabolites with other substituents (Meerts et al., 2000, 2001; Cantón et al., 2005, 2008; Harju et al., 2007), possibly due to the role of the —OH group as a hydrogen donor or acceptor (Harju et al., 2007). However, no consistent relationships between —OH group position and steroidogenic effects were found in either the present study or in previous studies (Cantón et al., 2005, 2008). For example, both the tetrabrominated 2′-OH-BDE-68 and 5-OH-BDE-47 have an —OH group adjacent to a bromine atom, but 2′-OH-BDE-68 induced the expression of several genes at 0.025 to 0.5 μM in the current study, while 5-OH-BDE-47 had no effect. Similar inconsistent effects have been reported for methoxylated PBDE metabolites; exposure to 10 μM 2′-MeO-BDE-68 significantly induced aromatase activity in the H295R cells in a previous study, but exposure to 5-MeO-BDE-47 did not (He et al., 2008). It is possible that the reason that structural relationships are difficult to determine for these compounds lies in differences in their metabolism, as some PBDE metabolites may be degraded within minutes while others are more persistent (Harju et al., 2007). Some hydroxylated PBDEs have also been shown to inhibit estradiol sulfotransferase activity, which could contribute to and prolong their endocrine effects (Harju et al., 2007).

In the current study, none of the tested chemicals significantly affected sex hormone production at any of the exposure concentrations tested, and some compounds had inconsistent or sometimes conflicting effects on different endpoints of the steroidogenic pathway. The lack of effects at the hormone level may be due to the relatively low exposure concentrations tested. Sex hormone concentrations are potentially the most functional endpoint measured in the present study, because increased hormone concentrations *in vivo* are more likely to have effects than elevated enzyme activity, which might change quickly. These results indicated that these low levels of PBDEs and their derivatives may pose little risk *in vivo*, but further studies are clearly needed to address this question.

In conclusion, the present study demonstrated that some OH— and MeO-BDEs are able to induce or inhibit steroidogenic gene expression and aromatase activity in the H295R human adrenocortical carcinoma cell line at environmentally relevant concentrations. The position of the OH-group with regard to neighboring bromine atoms may play a key role in the alteration of the expression of these genes and enzyme activities, but clear relationships between structure and toxicity could not be determined. Further research is needed to more fully understand the effects of PBDE metabolites and derivatives on the steroidogenic process.

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