Hydroxylated Metabolites of the Polybrominated Diphenyl Ether Mixture DE-71 Are Weak Estrogen Receptor-α Ligands

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BACKGROUND: Polybrominated diphenyl ethers (PBDEs) are widely found in the environment and are suspected endocrine disruptors. We previously identified six hydroxylated metabolites of PBDE (OH-PBDEs) in treated mice.

OBJECTIVE: We tested the hypothesis that OH-PBDEs would interact with and alter activity of estrogen receptor-α (ER-α).

METHODS: We tested estrogenicity using two assays: [3H]-estradiol ([3H]-E2) displacement from recombinant ER-α, and induction of reporter gene (ERE-luciferase) in cultured cells. We incubated the PBDE mixture DE-71 with rat liver microsomes and tested the resultant metabolite mixture for estrogenic activity. We also determined relative estrogenic potential of individual hydroxylated PBDE congeners.

RESULTS: Reporter gene activity was increased by DE-71 that had been subjected to microsomal metabolism. DE-71 did not displace E2 from ER-α, but all six of the OH-PBDE metabolites did. para-Hydroxylated metabolites displayed a 10- to 30-fold higher affinity for ER-α compared with ortho-hydroxylated PBDEs, and one produced a maximal effect 30% higher than that produced by E2. Coadministration of E2 and DE-71, or certain of its metabolites, yielded reporter activity greater than either chemical alone. Two ortho-OH-PBDEs were antiestrogenic in the reporter assay.

CONCLUSIONS: The observations—that the DE-71 mixture did not displace [3H]-E2 from ER-α, while the hydroxylated metabolites did—suggest that the weak estrogenic effects of DE-71 are due to metabolic activation of individual congeners. However, the behavior of DE-71 and its metabolites, when co-administered with E2, suggest a secondary, undetermined mechanism from classical ER-α activation.

KEYWORDS: cytochrome P450, DE-71, endocrine disruptors, ERE-luciferase, estrogens, mice, ovariectomized, PBDEs, polybrominated diphenyl ethers. Environ Health Perspect 116:1315–1321 (2008). doi:10.1289/ehp.11343 available via http://dx.doi.org/ [Online 27 May 2008]

DE-71 is a commercial mixture of mostly tetra- and penta-brominated diphenyl ethers (PBDEs), which has been used extensively as a flame retardant (Agency for Toxic Substances and Disease Registry 2004). DE-71 and other similar commercial mixtures (known collectively as pentaBDE) were used almost exclusively as flame retardants in flexible polystyrene foam, a major component of bed mattresses and upholstered products. Production of DE-71 ceased in 2004 (U.S. Environmental Protection Agency 2006), the same year that use of pentaBDE and other PBDE commercial mixtures was banned by the European Union (2003).

PBDEs are very stable compounds, and they are not chemically bonded to the material they are intended to protect from burning. As a result, they are widely found in environmental media (Hites 2004; Law et al. 2006) and can be found in human blood and milk (Furst 2006; Comaara et al. 2007; Lind et al. 2003; Main et al. 2007; Mazdai et al. 2003; Schecter et al. 2003). Some of the PBDE congeners most commonly found in human samples are BDE-47, BDE-99, and BDE-153 (Hites 2004; Comaara et al. 2007; Main et al. 2007; Mazdai et al. 2003). Two of these same congeners, the tetrabrominated BDE-47 and the pentabrominated BDE-99, are the main components of DE-71 (36% and 44% by weight, respectively); the hexabrominated BDE-153 is a minor component of DE-71 (4% by weight) (Qiu et al. 2007).

The prevalence of PBDEs in human tissue is of concern because these compounds are known to alter behavior, thyroid-hormone signaling, and sexual development in animals. Eriksson and colleagues found permanent aberrations in spontaneous behavior in rodents after developmental exposure to BDE-47, BDE-99, and BDE-153 (Eriksson et al. 2001; Eriksson et al. 2006; Viberg et al. 2004), or BDE-153 (Viberg et al. 2003). Serum thyroxine (T4) was significantly decreased in several different experimental models and by different pentaBDE mixtures: in rats exposed to DE-71 either prenatally or postnatally (Ellis-Hutchings et al. 2006; Stoker et al. 2004; Zhou et al. 2002); in American kestrels (Falco sparverius) exposed in ovo to a mixture of BDE-47, BDE-99, BDE-100 and BDE-153 (Fernie et al. 2005); and in adult female rats exposed to the commercial pentaBDE mixture Bromkal 70-5 DE (Darnert et al. 2007). The effect of PBDEs on T4 levels may require metabolic activation because hydroxylated PBDEs, but not the non-hydroxylated congeners, are able to bind human transthyretin in vivo (Meerts et al. 2000). Other effects of pentaBDE mixtures or their congeners in experimental animals suggest estrogenic or antiandrogenic activity. In rats, developmental exposure to BDE-99 affected the regulation of estrogen target genes (Ceccatelli et al. 2006), impaired spermatogenesis (Kuriyama et al. 2005), and decreased circulating sex steroids and reduced anogenital distance in males (Lilienthal et al. 2006). Male rats exposed to DE-71 on postnatal days 23–53 had reduced seminal vesicle and ventral prostate weights and delayed puberty (Stoker et al. 2004).

PBDEs are suspected to behave as estrogens because of the similarity of their chemical structures and properties to other xenosterogens, mainly the polychlorinated biphenyls (PCBs) (Hooper and McDonald 2000; Meerts et al. 2001; Pijnenburg et al. 1995). We have shown that DE-71 has weak estrogenic activity in vivo and in vitro (Mercado-Feliciano and Bigsby 2008). Because hydroxylated metabolites of a structurally similar class of halogenated aromatic pollutants, the PCBs, exert estrogenic effects (Carpenter 2006; Valharia and Gierthy 2000), it may be reasonable to expect that hydroxylated forms of PBDEs would also be estrogenic. Others have shown that some PBDE congeners and certain synthetically hydroxylated congeners could exert estrogenic effects in cultured cells (Hamers et al. 2006; Meerts et al. 2001). In a recent in vivo study, BDE-47 had uterotrophic effects in immature rats (Dang et al. 2007), suggesting in vivo activation of this otherwise nonestrogenic PBDE (Meerts et al. 2001).

We previously reported that DE-71 is metabolized in the mouse to produce hydroxylated metabolites (Qiu et al. 2007) and that it had mild estrogenic activity in the same animals (Mercado-Feliciano and Bigsby 2008). We conclude that the weak estrogenic activity we demonstrated is the result of the hydroxylated products of DE-71.

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could induce estrogenic signaling though ERE-luciferase induction by microsomal Figure 1. Test chemicals.

**Materials and Methods**

**Test chemicals.** We purchased dimethyl sulfoxide (DMSO) and estradiol [1,3,5(10)-estra
dione-3,17β-diol; E2] from Sigma Chemical Co. (St. Louis, MO). The PBDE congener mixture DE-71 was a gift from the Great Lakes Chemical Corporation (West Lafayette, IN); the congener composition was previously described by Qiu et al. (2007). The individual hydroxylated metabolites of PBDE [4-OH-
2,2’,4,4´-tetrabromodiphenyl (4´-OH-BDE-17); 2´-O-H-2,4,4´-tetrabromodiphenyl (2´-HO-BDE-28); 4-HO-2,2’,3,4´-tetrabromodiphenyl (4-OH-BDE-42); 3-OH-
2,2’,4,4´-tetrabromodiphenyl (3-OH-BDE-47); 6-OH-2,2’,4,4´-tetrabromodiphenyl (6-OH-BDE-47); and 4´-OH-2,2’,4,5´-tetrabromodiphenyl (4´-OH-BDE-49)] were synthesized as described by Marsh et al. (2004) and were gifts from G. Marsh (Stockholm University, Stockholm, Sweden). We purchased the brominated phenols 2,4-dibromophenol (2,4-DBP) and 2,4,5-tribromophenol (2,4,5-TBP) from Cambridge Isotope Laboratories (Cambridge, MA), DMSO was used as primary solvent for all chemicals, and the DMSO solutions were further diluted in cell culture media for treatments.

**Cells and culture conditions.** MDA-MB-231 breast cancer cells (Cailleau et al. 1978) obtained from ATCC (American Type Culture Collection; Manassas, VA) and BG1LucE2 ovarian cancer cells, a gift from M. Denison (University of California, Davis, CA), were used in estrogen bioassays. BG1LucE2 cells are BG-1 ovarian cancer cells (Geisinger et al. 1989) stably transfected with an estrogen-responsive plasmid (Rogers and Denison 2000). Most cell culture media and supplements were purchased from Gibco/ Invitrogen (Carlsbad, CA), except bovine growth serum (BGS; Hyclone, Logan, UT) and E2 (Sigma). Most charcoal-strip
ing reagents and endotoxin-free water were purchased from Sigma-Aldrich (St. Louis, MO) except Dulbecco’s phosphate-buffered saline (DPBS; Mediatech Inc., Herndon, VA). MDA-MB-231 cells were maintained in growth medium (GM): minimum essential media (MEM) supplemented with t-glutamine (2 mM), nonessential amino acids (0.1 mM), HEPES buffer (10 mM), 0.4 pg/mL insulin, and 5% vol/vol BGS. BG1LucE2 cells were maintained in BG1-GM: alpha-MEM supplemented with HEPES buffer (10 mM), geneticin (0.4 g/L) and 10% vol/vol BGS. Basal medium (BM) for MDA-MB-231 cells consisted of a formulation similar to GM, except that phenol red-free MEM and 3% charcoal
collected BM.DMSO mixture were further diluted in cell culture media for treatments.

**ER-negative MDA-MB-231 breast cancer cells were plated in BM. Two days later cells were transfected using Tfx-20 (Promega, Madison, WI) and 0.6 nM recombi
ner ER-α (H-E2; Amersham Biosciences, Buckinghamshire, UK) and 0.6 nM recombi
ner ER-α (Panvera/Invitrogen, Madison, WI) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) at 4°C overnight. Hydroxylationate (60% in TE buffer) was added, mixed well, and incubated for 15 min at room tempera
ture. The resulting slurry was washed three times by centrifugation at 3,000 RCF at 4°C for 1 hr to remove microsomes. The hydroxylated organic fraction was extracted from the supernatant by solid-state extraction with ethanol elution using Sep-Pack Plus C18 cartridges (Waters Corp.; Milford, MA), then evaporated to dryness in vacuo and reconstituted in a volume of DMSO that would yield 10 mM PBDE or 10 µM estradiol, assuming 100% recovery. This extraction procedure was adapted from Yoshihara et al. (2004).

**Recombinant ERα binding assay.** Vehicle or test chemicals were incubated with 1 nM tritiated E2 (3H-E2; Amersham Biosciences, Buckinghamshire, UK) and 0.6 nM recombi
ner ER-α (Panvera/Invitrogen, Madison, WI) in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) at 4°C overnight. Hydroxylapatite (60% in TE buffer) was added, mixed well, and incubated for 15 min at room tempera
ture. The resulting slurry was washed three times by centrifugation at 3,000 RCF at 4°C for 1 hr to remove microsomes. The hydroxylated organic fraction was extracted from the supernatant by solid-state extraction with ethanol elution using Sep-Pack Plus C18 cartridges (Waters Corp.; Milford, MA), then evaporated to dryness in vacuo and reconstituted in a volume of DMSO that would yield 10 mM PBDE or 10 µM estradiol, assuming 100% recovery. This extraction procedure was adapted from Yoshihara et al. (2004).

**Figure 1.** ERE-luciferase induction by microsomal metabolites of DE-71. Incubations with an incomplete NADPH generating system (lacking NADP+) were run in parallel and served as the negative control. The incubation products were tested in ERE-luciferase assays. One representative assay is shown; results are presented as mean ± SD (n = 4). The results are representative of three similar assays.

**Table 1.** Blood serum concentrations [µM (mean ± SE)] of phenolic metabolites found in mice after DE-71 treatment.

| Compound            | Vehicle control | DE-71 |
|---------------------|-----------------|--------|
| 2,4-DBP             | 0.01 ± 0.02     | 0.29 ± 0.09 |
| 2,4,5-TBP           | 0.001 ± 0.002   | 0.44 ± 0.02 |
| 4′-OH-BDE-17        | 0.04 ± 0.04     | 0.04 ± 0.02 |
| 2′-O-H-BDE-28       | 0.0002 ± 0.0005 | 0.03 ± 0.01 |
| 4-hydroxy-BDE-28    | 0.0002 ± 0.0005 | 0.03 ± 0.01 |
| 3-OH-BDE-47         | ND              | 0.11 ± 0.05 |
| 6-OH-BDE-47         | ND              | 0.04 ± 0.02 |
| 4′-OH-BDE-49        | 0.001 ± 0.002   | 0.08 ± 0.04 |
| DE-71+              | <0.02           | 3.8 ± 0.6  |

ND, not detected. Mice were treated with 50 mg/kg/day DE-71 (per g) for 34 days. Concentrations were determined by gas chromatographic mass spectrometry analy
sis. Modified from Qiu et al. (2007).

**Statistics.** All statistics were performed using GraphPad Prism, version 3.0a, for Macintosh (GraphPad Software, San Diego CA). For each analysis, we determined whether groups had unequal variances by Bartlett’s test. Group averages with equal vari
ances were compared to each other either by...
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one-way analysis of variance (ANOVA) with Tukey post-test or by unpaired $t$-test as appropriate. Group averages with unequal variances were compared to each other by $t$-test with Welch’s correction. Groups treated with DE-71 or E2 alone were compared with controls treated with DE-71 alone. All values are expressed as mean ± SD or SE as indicated. We considered groups statistically different if $p < 0.05$ by ANOVA with Tukey posttest or $t$-test (two-tailed). Dose–response studies were also subjected to regression analysis using a sigmoidal curve fitting model:

$$\text{Response} = \min + \left(\frac{[\max - \min]}{1 + 10^{[\log(EC_{50}) - \log(dose)]} + \text{Hillslope}}\right),$$

where $EC_{50}$ is the median effective concentration. The modeled curve is shown in figures if $R^2 > 0.8$.

**Results**

**In vitro microsomal metabolism increases estrogenic character of DE-71.** To determine whether microsomal metabolism could increase estrogenic activity, we incubated DE-71 with female rat microsomes, with or without a complete NADPH generating system. The incubation product was then tested in ERE-luciferase assays, using either the transient transfection system with MDA-MB-231 cells or the stably transfected ER-α–positive BG1Luc4E2 cells. At $10^{-5}$ M, DE-71 induced little ERE-luciferase activity when it was incubated in the buffer lacking an NADPH generating capacity (without NADP+; negative control), but the product of the complete incubation buffer produced a 4-fold increase in luciferase (Figure 1). Microsomal incubation decreased the activity of E2 by 78%, an expected result because hydroxylation decreases the activity of E2 (Martucci and Fishman 1993). Furthermore, the level of activity of the extract from the E2 incubated without NADP+ indicates that recovery of compound by the extraction methods used and in three separate microsomal incubation experiments was 66–98%.

**Phenolic metabolites found in blood of mice after treatment with DE-71.** In a previous study (Qu et al. 2007), we examined blood serum from BALB/c mice that had been treated with DE-71 for 34 days and quantified identifiable phenolic metabolites that included two brominated phenols and six hydroxylated brominated diphenyl ethers (Table 1). These findings raise the possibility that the estrogenic effects seen earlier in mice and in culture (Mercado-Feliciano and Bigsby 2008) were due to the metabolites and not necessarily the original DE-71 congeners.

**DE-71 and its phenolic metabolites activate the ERE.** E2 induces ERE-luciferase at concentrations above $10^{-13}$ M in the stably transfected BG1Luc3E2 reporter cell culture system (Figure 2), with an $EC_{50}$ in the picomolar range (Table 2). Next DE-71 and the metabolites found in mice were tested using BG1Luc3E2 cells to determine if they were able to activate ERE-mediated gene transcription (Figure 2), and their potencies and effectiveness were compared to that of E2 (Table 2). The potencies of each test compound were estimated by determining the compound’s own $EC_{50}$ from its own maximal effect, and also by calculating its relative estrogenic potency determined from the concentration required to produce an effect equivalent to E2’s $EC_{50}$.

DE-71 was able to significantly induce ERE-luciferase at test concentrations $≥ 5 \times 10^{-5}$ M, reaching the same effectiveness as E2 at $10^{-4}$ M (Figure 2B). The calculated $EC_{50}$ for DE-71 was $3.7 \times 10^{-5}$ M. Because the maximal effect of DE-71 was very close to the maximal effect of E2, its $EC_{50}$ ($3.7 \times 10^{-5}$ M) and its estrogen equivalency potency (EEP;...
3.9 × 10⁻⁵ M) were similar. One metabolite, 4'-OH-BDE-17 was clearly more potent than DE-71, with an EC₅₀ in the micromolar range (Table 2). 4'-OH-BDE-17 had a relative estrogenic potency approximately 10-fold that of DE-71, and it was more effective than DE-71 or E₂, reaching an estimated maximal effect 30% higher than E₂ (Figure 2C; Table 2). 4'-OH-BDE-49 had an EC₅₀ similar to that of DE-71 but a much lower efficacy; its maximal effect did not even reach the EC₅₀ for E₂ (Figure 2E; Table 2). Another para-hydroxylated metabolite, 4'-HO-BDE-42, appears to have been more potent than DE-71, but because of limited availability of this compound, the analysis was not carried out with a sufficient span of concentrations to allow an accurate estimate of the EC₅₀ (Figure 2D).

**DE-71 phenolic metabolites displace ³H-E₂ from ER-α.** We assessed the ability of the metabolites found in mouse blood to displace ³H-E₂ from recombinant ER-α, and results are summarized in Figure 3 and Table 3. Neither the DE-71 mixture nor the bromophenol metabolites 2,4-DBP and 2,4,5-TBP were able to displace ³H-E₂ from ER-α (Figure 3B,I,J). All of the hydroxylated BDEs displaced ³H-E₂ from ER-α, but their relative binding affinities were very low (Table 3). Of the OH-BDEs tested, the para-hydroxylated congeners (at either the 4 or 4’ position) had a higher affinity for the estrogen receptor than 2-, 3-, or 6-OH-BDEs. 4'-OH-BDE-17 and 4'-OH-BDE-49 were the most potent, with mean inhibitory concentrations (IC₅₀) in the micromolar range (Figure 3C,D,E; Table 3). 6-OH-BDE-47, 6-OH-BDE-47,

### Table 2. Potency and efficacy estimates of DE-71 metabolites in the ERE-luciferase assay.

| Compound     | EC₅₀ (M) | EEP (M) | Relative estrogen potency (ratio) | Fold induction at EC₅₀ | Relative effect (ratio) |
|--------------|----------|---------|----------------------------------|-----------------------|-------------------------|
| E₂           | 1.2 × 10⁻¹² | 1.00    | 3.3                              | 1.00                  |
| DE-71        | 3.7 × 10⁻⁵  | 3.9 × 10⁻⁵ | 3.1 × 10⁻⁸                       | 3.4                   |
| 2,4-DBP      | No effect | NR      | —                                | —                     |
| 2,4,5-TBP    | No effect | NR      | —                                | —                     |
| 4'-OH-BDE-17 | 4.7 × 10⁻⁶  | 3.5 × 10⁻⁶ | 3.4 × 10⁻⁷                       | 4.3                   |
| 2'-OH-BDE-28 | NA       | NA      | —                                | —                     |
| 3-OH-BDE-47  | NA       | NA      | —                                | —                     |
| 6-OH-BDE-47  | No effect | NR      | —                                | —                     |
| 4'-OH-BDE-49 | 1.3 × 10⁻⁵  | 1.5 × 10⁻⁵ | 0.001                           | 0.36                  |

Abbreviations: NA, not available because effect was insufficient to calculate an EC₅₀; NR, E₂ EC₅₀ not reached. All values were estimated from curves derived in Figure 2.

* Determined using the chemical’s own maximum effect set at 100%.
* Concentration inducing the same luciferase activity as the EC₅₀ of E₂.
* Ratio of the E₂ EC₅₀ to EEP.
* Test chemical-to-E₂ ratio of luciferase induction at EC₅₀.

### Table 3. ER-α relative binding affinities.

| Compound     | IC₅₀ (M) | Relative affinity (%) |
|--------------|----------|-----------------------|
| E₂           | 6.9 × 10⁻¹⁰ | 100                   |
| DE-71        | No effect | —                     |
| 2,4-DBP      | No effect | —                     |
| 2,4,5-TBP    | No effect | —                     |
| 4'-OH-BDE-17 | 2.1 × 10⁻⁶  | 0.03                  |
| 2'-OH-BDE-28 | 6.0 × 10⁻⁵  | 0.001                 |
| 3-OH-BDE-47  | 5.2 × 10⁻⁵  | 0.001                 |
| 6-OH-BDE-47  | 1.1 × 10⁻⁵  | 0.001                 |
| 4'-OH-BDE-49 | 2.3 × 10⁻⁶  | 0.03                  |

* The concentration of test compound yielding 50% displacement of ³H-E₂ from receptor, calculated based on data shown in Figure 3.

**Figure 3.** Displacement of 1 nM ³H-E₂ from recombinant ER-α in vitro by E₂ (A), DE-71 (B), and the OH-BDEs 4'-OH-BDE-17 (C), 4'-OH-BDE-42 (D), 4'-OH-BDE-49 (E), 2'-OH-BDE-28 (F), 3-OH-BDE-47 (G), 6-OH-BDE-47 (H), 2,4-DBP (I), and 2,4,5-TBP (J) found in mice (by the ER-α binding assay). Each curve for an OH-BDE represents the mean of 3-4 independent dose–response studies, except for 4'-OH-BDE-49, 6-OH-BDE-47, and the bromophenols only 2 assays were performed. Curves for DE-71 and E₂ are the mean of 6 and 11 independent dose–response studies, respectively. Error bars indicate SE. All values are normalized to vehicle control (DMSO = 1). Modeled data for E₂ (dashed blue line) and DE-71 (dotted line) are shown in all OH-BDE charts for comparison. Modeled data for each OH-BDE are shown as a solid line.
DE-71 phenolic metabolites modify ERE activation by $E_2$. Because DE-71 and several of its hydroxylated metabolites were able to either activate and/or displace $^3$H-$E_2$ from ER-$\alpha$, we cotreated BG1Luc3E2 cells with $10^{-11}$ M $E_2$ and the specified chemical; data are normalized to the maximal effect of $E_2$ (set at 1.0). Each curve is the mean of 3–5 independent dose–response studies, and dashed line) are shown in all charts for comparison.

**Figure 4.** ERE-luciferase induction in BG1Luc4E2 cells after coteatment with $E_2$. See “Material and Methods” for details. Squares indicate data for cotreatment with $E_2$ and the specified chemical; data are normalized to the maximal effect of $E_2$ (set at 1.0). Each curve is the mean of 3–5 independent dose–response studies, except for 2-OH-BDE28, 6-OH-BDE47, and 4-OH-BDE49 for which only 2 assays were performed. Error bars indicate SE. Modeled data for $E_2$-only dose–response (blue dashed line) are shown in all charts for comparison.

* $p > 0.5$, ** $p < 0.01$, and *** $p < 0.001$ compared with the maximal effect of $E_2$ alone.

3-OH-BDE-47, and 2′-OH-BDE-28 had IC$_{50}$ values one order of magnitude higher than the para-OH BDEs (Figure 3F,G,H; Table 3). In general, the potency of each OH BDE displacing $^3$H-$E_2$ from ER-$\alpha$ correlates with their ability to activate ERE-luciferase, and the congeners with the highest IC$_{50}$ values induce very little (3 OH-BDE-47) or no significant ERE-luciferase activity (2′-OH-BDE-28 and 6-OH-BDE-47).

**Table 3**

| Chemical        | IC$_{50}$ (nM) |
|-----------------|----------------|
| OH-PBDE tested  |                |
| 3-OH-BDE-47     |                |
| 4-OH-BDE-42     |                |
| 2′-OH-BDE-28    |                |
| 6-OH-BDE-47     |                |

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Because DE-71 and several of its hydroxylated metabolites were able to either activate and/or displace $^3$H-$E_2$ from ER-$\alpha$, we cotreated BG1Luc3E2 cells with $10^{-11}$ M $E_2$ and the specified chemical; data are normalized to the maximal effect of $E_2$ (set at 1.0). Each curve is the mean of 3–5 independent dose–response studies, except for 2-OH-BDE28, 6-OH-BDE47, and 4-OH-BDE49 for which only 2 assays were performed. Error bars indicate SE. Modeled data for $E_2$-only dose–response (blue dashed line) are shown in all charts for comparison.

* $p > 0.5$, ** $p < 0.01$, and *** $p < 0.001$ compared with the maximal effect of $E_2$ alone.
proestrogen methoxychlor (Bulger et al. 1978; Kupfer and Bulger 1979). Preincubation of DE-71 with microsomes under enzyme-activating conditions increased its estrogenic activity. Mammalian liver microsomes are rich in cytochrome P450 (CYP450), a group of isoenzymes responsible for metabolism of many endogenous and exogenous chemicals including estrogens (reviewed by Bigsby et al. 2005). The biological activities of environmental chemicals have been found to be either increased or decreased by specific CYP450s (Goldstein and Faletto 1993), and some of these chemicals are known to be converted into estrogens by CYP450 metabolism (Bulger et al. 1978; Kohno et al. 2005; Kupfer and Bulger 1979; Morohoshi et al. 2005). Based on findings by Qui et al. (2007) and others (Malmberg et al. 2005; Marsh et al. 2006), the DE-71 congener BDE-47 seems to be the source of activated OH-PBDEs in laboratory rodents; BDE-47 itself has been found to have little estrogenic activity (Hamers et al. 2006; Meerts et al. 2001). Others have also found OH-PBDEs in wild marine animals that could be BDE-47 metabolites (Kelly 2006; Verreault et al. 2005; Verreault et al. 2007). However, the source of OH-PBDEs in the marine environment can be both natural and anthropogenic, because some marine organisms produce natural brominated compounds (Vetter 2006).

We observed both estrogenic and antiestrogenic effects in our study. We found that metabolites of DE-71 hydroxylated at the ortho position could act as antiestrogens. Although cotreatment with DE-71 produced a larger effect than the maximal response to E2 in the present study using a reporter gene assay, in effect than the maximal response to E2 

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\text{E}_2
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metabolites of DE-71 hydroxylated at the estrogenic effects in our study. We found that either estrogenic or antiestrogenic activity in culture also inhibit estrogen sulfotransferases (E2SULT) in vitro. Such an effect would translate into an increased activity of administered E2 and could explain the ability of 3-OH-BDE-47 to increase ERE-luciferase expression above the level induced by E2 alone when BG-1Luc4E2 cells are cotreated with both chemicals, even though 3-OH-BDE-47 does not induce ERE-luciferase by itself. However, the E2 dose used in cotreatment with 3-OH-BDE-47 was already high enough to reach the maximal effect in this system; therefore, it is unlikely that making more E2 available by inhibiting E2SULT would increase ERE-luciferase signaling. Furthermore, it is not known whether the BG-1Luc4E2 cells express E2SULT, and we observed no significant potentiation of the estrogenic effect by 4-OH-BDE-42, another metabolite that behaves as a potent inhibitor of E2SULT in vitro. A weaker E2SULT inhibitor, 6-OH-BDE-47, actually produced an antiestrogenic effect in our system. Thus, it is unlikely that altered E2SULT activity explains the additional estrogenic activity produced by 3-OH-BDE-47.

The levels of DE-71 needed to have estrogenic effects in our studies (micromolar range) are much higher than the highest concentrations found so far in human blood serum (0.1–5 nM; Mazdaii et al. 2003). However, there is little current information on the levels of OH-PBDEs in human serum or the role that human enzymes (especially CYP450) may play in the formation of DE-71 metabolites. Rodent tissues do not have the same CYP450 activities as human tissues (Bogaards et al. 2000); therefore, the metabolites we found in mouse serum (Qui et al. 2007) or those found by Marsh et al. (2006) in rat feces may not be representative of metabolites formed in humans. Several of the PBDE metabolites we found in mice (4′-OH-BDE-17, 6-OH-BDE-47, 3-OH-BDE-47, and 4-OH-BDE-42) have been found in human serum samples from children (4′-OH-BDE-17, 6-OH-BDE-47, 3-OH-BDE-47, and 4-OH-BDE-42) have been found in human serum samples from children. These metabolites are produced by individual BDE congeners from the sum of estrogenic and antiestrogenic activities of metabolites. Compound may act as endocrine disruptors through a number of mechanisms, including indirectly by altering metabolism of endogenous hormones. Hamers et al. (2008) found that BDE-47 and several of the OH-PBDE metabolites that we found to have either estrogenic or antiestrogenic activity in culture also inhibit estrogen sulfotransferases (E2SULT) in vitro. Such an effect would translate into an increased activity of administered E2 and could explain the ability of 3-OH-BDE-47 to increase ERE-luciferase expression above the level induced by E2 alone when BG-1Luc4E2 cells are cotreated with both chemicals, even though 3-OH-BDE-47 does not induce ERE-luciferase by itself. However, the E2 dose used in cotreatment with 3-OH-BDE-47 was already high enough to reach the maximal effect in this system; therefore, it is unlikely that making more E2 available by inhibiting E2SULT would increase ERE-luciferase signaling. Furthermore, it is not known whether the BG-1Luc4E2 cells express E2SULT, and we observed no significant potentiation of the estrogenic effect by 4-OH-BDE-42, another metabolite that behaves as a potent inhibitor of E2SULT in vitro. A weaker E2SULT inhibitor, 6-OH-BDE-47, actually produced an antiestrogenic effect in our system. Thus, it is unlikely that altered E2SULT activity explains the additional estrogenic activity produced by 3-OH-BDE-47.

In summary, the observations that the DE-71 mixture does not displace [3H-E2 from ER-α—while the hydroxylated metabolites do—suggest that the weak estrogenic effects of DE-71 are due to metabolic activation of individual congeners. However, the behavior of DE-71 and some of its metabolites when coadministered with E2 suggest a secondary, undetermined mechanism of action different from classical ER-α activation.

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