The polybrominated diphenyl ether mixture DE-71 is mildly estrogenic
Minerva Mercado-Feliciano¹ and Robert M. Bigsby²

¹Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana, USA

BACKGROUND: Polybrominated diphenyl ethers (PBDEs) are widely found in the environment, and they may act as endocrine disruptors.

OBJECTIVE: Our goal in this study was to test the PBDE mixture DE-71 for estrogenic activity.

METHODS: We used proliferation of cultured breast cancer cells (MCF-7) and trophic effects in the reproductive tracts of ovariectomized mice as estrogen bioassays. DE-71 was administered to mice by subcutaneous injection (sc) or oral gavage (po), alone or in combination with estradiol, for 3 or 34 days. Liver weights and cytochrome P450 enzyme activities were also measured.

RESULTS: DE-71 increased MCF-7 cell proliferation, and this was prevented by antiestrogen. DE-71 cotreatment reduced the effect of estradiol in MCF-7 cells. In the mouse 3-day assay, DE-71 administered alone had no effect on uterine weight, uterine epithelial height (UEH), or vaginal epithelial thickness (VET); however, when DE-71 was administered as a cotreatment, it potentiated estradiol’s effect on uterine weight. DE-71 administered sc to BALB/c mice for 34 days slightly increased UEH and VET, and attenuated the estradiol-induced increase in UEH; these effects were not seen in BALB/c mice treated po or in C57BL/6 mice treated sc. DE-71 increased liver weight in BALB/c, C57BL/6, and estrogen receptor-α (ERα) knockout mice. We also found an increase in liver cytochrome P450 1A (CYP1A) and CYP2B activities when DE-71 was administered po, but only CYP2B increased after sc treatment.

CONCLUSION: DE-71 behaves as a weak estrogen. In mice, the treatment route and duration determined if DE-71 was estrogenic. BALB/c mice are more susceptible to DE-71 effects in estrogen target tissues than C57BL/6 mice. DE-71 increased liver weight independently of estrogen receptor-α.

KEY WORDS: CYP1A, CYP2B, DE-71, endocrine disruptors, estrogens, MCF-7, mice, ovariectomy, PBDEs, polybrominated diphenyl ethers. Environ Health Perspect 116:605–611 (2008). doi:10.1289/ehp.10643 available via http://dx.doi.org [Online 29 January 2008]
was not tested for furan content (Hites R, Qiu X, personal communication). DMSO was used as the primary solvent for all treatment chemicals, and the DMSO solutions were further diluted in corn oil for animal treatments. DE-71 remained in solution in DMSO at all doses used, but vials were still vigorously vortexed for several seconds immediately before treatment. DE-71 prepared for in vivo treatments was maintained in suspension in corn oil by continuous stirring while the needle was filled, and animals were dosed immediately.

Cell proliferation measurements. To measure cell proliferation, we used the colorimetric assay developed by Mosmann (1983) with minor modifications. Cells were regularly maintained in growth medium (i.e. minimum essential media (MEM; Gibco/Invitrogen; Carlsbad, CA) supplemented with t-glutamine (2 mM; Gibco/Invitrogen), nonessential amino acids (0.1 mM; Gibco/Invitrogen), HEPES buffer (10 mM; Gibco/Invitrogen), and 5% vol/vol bovine growth serum (Hyclone, Logan, UT)). To minimize basal hormonal activity during assays, cells were incubated in basal medium (i.e., phenol red free MEM supplemented with 2 mM t-glutamine, 0.1 mM nonessential amino acids, 10 mM HEPES, and 3% vol/vol dextran-coated charcoal-stripped bovine growth serum).

For the cell proliferation assay, cells cultured with growth medium were plated in 24-well dishes (15,000 cells/well). The day after plating, culture medium was changed to basal medium. Starting 2 days after changing to basal medium, cells were cultured in treatment medium (basal medium plus treatment) for a total of 10 days, changing the treatment medium every 2 days. On day 10, cells were incubated in tetrazolium MTT [3-(4,5-dimethylthiazolyl-2),5-diphenyltetrazolium bromide] for 2 hr, then lysed in acid isopropanol. Reduced (blue) MTT absorbance was read at 570 nm.

To confirm the MTT absorbance correlated with cell growth and not increased metabolic activity, in some experiments we included additional plates treated in parallel for DNA determination. After 10 days of treatment, cells were washed twice with phosphate-buffered saline and incubated for 10 sec in cold methanol. After removing the methanol, we allowed cells to dry at room temperature. Cells were then dissolved in 0.5 M sodium hydroxide by rocking in a humidified 37°C chamber for 30 min. Samples were then transferred to microtubes and incubated at 65°C for 1 hr. Sample (100 µL) was diluted in TNE buffer (10 mM Tris, 0.1 M NaCl, 1 mM EDTA, pH 7.4) containing 0.1 mg/mL Hoechst 33258 dye (PolySciences, Warrington PA), and neutralized with an equimolar amount of hydrochloric acid. Fluorescence was measured in a Hoefer TKO 100 DNA Fluorometer (Hoefer Scientific Instruments, San Francisco, CA) against a salmon sperm DNA standard (Invitrogen, Carlsbad, CA).

Animal treatments. All procedures performed on animals were approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine.

![Figure 1. DE-71 induced proliferation of MCF-7 cells treated for 10 days with DE-71 and/or E2 at the indicated concentrations. Proliferation was measured using the MTT assay (A, C, D) or a DNA assay (B). The antiestrogen fulvestrant (ICI, 10 nm) blocked the effect of DE-71 (C). DE-71 blocked the effect of E2 in a dose-dependent manner (D). Values shown are mean ± SE of three to seven independent assays. *p < 0.05, **p < 0.01, and ***p < 0.001 vs. vehicle; #p < 0.01, and ##p < 0.001 vs. treatment without ICI.](image-url)
We measured 7-ethoxyresorufin O-dealkylation (EROD; CYP1A) activity and 7-pentoxyresorufin O-dealkylation (PROD; CYP2B) activity by mixing 5 µL of sample with 5 µL 250 µM NADPH and 3.4 µL 0.6 mM 7-ethoxyresorufin or 7-pentoxyresorufin in 1.2 mL 0.1 Tris buffer at 37°C. After allowing the mixture to equilibrate for 1 min, fluorescence was measured at 530 nm excitation and 585 nm emission in a fluorometer at 1-s intervals over the course of 1 min. Similar measurements were made with six different resorufin concentrations to determine a standard curve. The slope of the linear range for each activity assay (fluorescence/sec) was converted to moles of resorufin per gram per second (mol/g·sec) using the standard curve and the protein content of each sample.

Statistics. All statistics were performed using GraphPad Prism, version 3.0a, for Macintosh (GraphPad Software, San Diego, CA). For each statistical analysis, we used Bartlett’s test to determine if groups had unequal variances. Group averages with equal variances were compared to each other by either one-way analysis of variance (ANOVA) with Tukey post-test or 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 alone were analyzed against vehicle controls. Groups cotreated with DE-71 and EB were analyzed against controls treated with EB alone. All values are expressed as mean ± SE. Groups were considered statistically different if the result of ANOVA with Tukey post-test or t-test (two-tailed) had p < 0.05; however, in some instances, means were considered of borderline statistical significance if 0.05 < p < 0.10. DE-71 dose–response studies were also subjected to regression analysis using three curve fitting models: a) linear: response = (slope × dose) + intercept; b) sigmoidal: response = minimum + (maximum – minimum) × [1 + 10^([Log(EC50) - Log(dose)])] × Hillslope; c) modified Gaussian distribution: response = minimum + ([maximum – minimum] × exp[-([log(dose) – A] + slope)]^2, where A = Log(EC50) + 0.833 × slope, and the EC50 is the median effective concentration. The best fitting curve appears in graphs if R² > 0.8.

Results

Cell proliferation assays. We compared the capacity of DE-71 to increase breast cancer cell (MCF-7) proliferation with that of E2. Both E2 and DE-71 were able to significantly increase cell number, as determined by the MTT assay (Figure 1A). DNA assays confirmed this finding, indicating that the increase in reduced MTT was caused by an increase in cell number and was not merely an effect on the cell redox systems (Figure 1B). The effects of both E2 and DE-71 were negated by cotreatment with the estrogen antagonist ICI (Figure 1C). DE-71 produced a biphasic dose–response curve, suggesting that it may have been toxic to MCF-7 cells at concentrations > 2.5 × 10^-5 M (Figure 1A). At those same concentrations, we observed an accumulation of a white precipitate, suggesting a solubility problem. Cotreatment of cells with both E2 and DE-71 resulted in a lesser increase in cell proliferation compared with E2 alone, suggesting an antagonistic effect of DE-71 on E2-induced cell proliferation (Figure 1D). Antagonism was dose dependent; the highest dose tested corresponds to that which produced maximal proliferative effect when cells were treated with DE-71 alone.

Three-day mouse estrogenic endpoints. We used a 3-day treatment regimen in OVX BALB/c mice to assess DE-71 effects at three doses (75, 150, and 300 mg/kg); groups of animals were also treated with 10 µg/kg EB plus DE-71 at each of these doses. Uterine wet weight, UEH, and VET were used as estrogenic end points. DE-71 administration by sc alone had no statistically significant effect (Figure 2). However, the increase in uterine wet weight induced by 10 µg/kg EB was enhanced by DE-71 in a dose-dependent manner; this enhanced response was equivalent to the maximal estrogen effect produced by 10 µg/kg EB (Figure 2B). Oral DE-71 administration had no effect on any of the estrogenic parameters measured (data not shown).

Subcutaneous DE-71 treatment alone had no effect on UEH or VET (Figure 2C, 2E). When mice were cotreated with DE-71 and EB, there was an increasing dose–response trend for UEH and VET (Figure 2D, 2F). However, only borderline statistical significance was achieved for UEH in the highest dose group, and neither trend yielded a good fit by regression analysis.

Thirty-four-day mouse estrogenic endpoints. We determined estrogenic effects in ovariectomized BALB/c and C57BL/6 WT and ER-α KO mice treated long-term (34 days). We examined uterine and vaginal parameters as in the 3-day assay. As with the 3-day assay, oral DE-71 administration had no effect on any of the estrogenic parameters.
measured in BALB/c mice (data not shown); C57BL/6 mice were not treated orally.

Treatment for 34 days with sc DE-71 alone produced no significant change in uterine weight (Figure 3A). There was a large increase (8- to 12-fold; \( p < 0.001 \)) in uterine weight of WT mice after \( E_2 \) treatment. The uterine weight response to 10 \( \mu \text{g/kg} \) EB at 34 days was similar to the increase produced by 10 \( \text{mg/kg} \) EB for 3 days; that is, the 34-day EB treatment produced the maximal uterotrophic effect. EB-induced uterine weight was unaffected by cotreatment with DE-71 (Figure 3A).

In BALB/c mice, DE-71 administered sc for 34 days caused a 23% increase in UEH (Figure 3C) and a 33% increase in VET (Figure 3E). When administered alone, DE-71 had no effect on these parameters in C57BL/6 mice. In contrast, we observed a small but statistically significant decrease in the estrogen-induced UEH increase in BALB/c mice cotreated with sc DE-71 (Figure 3D). The similar decrease in UEH in cotreated C57BL/6 mice was not statistically significant. Cotreatment did not alter the ER-\( \alpha \)-induced increase in VET (Figure 3F).

As expected, EB treatment had no effect on ER-\( \alpha \)-KO uterine weights, UEH, or VET, nor did DE-71 have an effect on any of the uterine or vaginal parameters in either WT C57BL/6 mice (Figure 3) or ER-\( \alpha \)-KO animals (data not shown).

Liver end points. We determined liver weights and activities of CYP1A and CYP2B enzymes. We found a 20–51% increase in liver weight in BALB/c mice treated with DE-71 for 34 days compared with vehicle control, whereas livers of 3-day treated mice increased in weight up to 39% in a dose-dependent manner (Figure 4).

EB treatment did not increase liver weight in BALB/c mice. However, EB potentiated the effect of po DE-71 but had no effect on sc DE-71 treatments (Figure 4B). As with BALB/c mice, the livers of DE-71 treated C57BL/6 WT mice were 27–29% larger than vehicle-treated controls. \( E_2 \) administered alone or in combination with DE-71 had no effect on liver weights of C57BL/6 WT mice. The size of ER-\( \alpha \)-KO mouse livers increased 30% after DE-71 treatment (Figure 4C).

We found a large increase in PROD activity in DE-71–treated BALB/c mice compared with controls, about 7-fold in the po-treated group and 5-fold in the sc-treated group (Figure 5A). Still, PROD activity in the DE-71–induced animals was much lower than PROD activity in vehicle-treated animals (i.e., the maximal PROD was about one-third the minimal PROD activity). Liver microsomal EROD activity also increased (2.5-fold), but only for po-treated mice (Figure 5B). EB treatment had no effect on either EROD or PROD activity (data not shown).

Discussion

DE-71 exhibited both estrogenic and antiestrogenic effects in the MCF-7 cell proliferation assay and in the adult ovariectomized mouse model, a behavior expected from a weak ER agonist (reviewed by Lerner and Jordan 1990). The observation that ICI prevented DE-71 from increasing cell number in the MCF-7 bioassay suggests the involvement of an ER.

To date, this is the first report of a study in which the estrogenic action of a PBDE mixture has been examined using standard rodent bioassay end points. The magnitude of the effects seen in our in vivo studies was similar to those produced by treatment of ovariectomized rats with 200 mg/kg BDE-47, a major component of DE-71 (Dang et al. 2007). The type of response observed (agonist or antagonist) was dependent upon the duration of exposure to DE-71. In the 3-day assay DE-71 administered alone produced no estrogenic effects; however, when it was administered for 34 days, it produced hypertrophy of the uterine epithelium and hyperplasia of the vaginal epithelium. When administered with EB in the 34-day treatment schedule, DE-71 enhanced the estrogen effect, whereas in the 34-day treatment schedule DE-71 produced small antiestrogenic effects. Thus, pharmacokinetic considerations are paramount when designing further studies of PBDE action in vivo.

We used the ER-\( \alpha \)-KO mouse to determine if in vivo effects of DE-71 were mediated by ER-\( \alpha \); however, because there was a lack of any estrogenic effects of DE-71 in C57BL/6 WT mice, this experiment was not informative. The observation that DE-71 could enhance estrogenic effects beyond those produced by a saturating dose of EB suggests that PBDEs modify estrogen action through nonclassical pathways, as has been proposed for other xenobiotics such as \( \beta \)-hexachlorocyclohexane (Hatakeyama et al. 2002; Steinmetz et al. 1996).

PBDEs are suspected to behave as estrogenic because of the similarity of their chemical structure and properties to other xenobiotics, mainly the polychlorinated biphenyls [reviewed by Hooper and McDonald (2000), Meerts et al. (2001), and Pijnenburg et al. (1995)]. Furthermore, hydroxylated metabolites of PCBs have been shown to exert estrogenic effects [reviewed by Bigby et al. (2005) and Blair et al. (2000)]; therefore, it may be reasonable to expect that hydroxylated forms of
PBDEs would also be estrogenic. Several researchers have shown that individual BDE congeners or certain synthetic hydroxylated congeners could exert estrogenic effects in cultured cells. In estrogen-responsive transcription reporter assays, BDE-28 and BDE-100, as well as the 4′-hydroxy forms of BDE-30 and BDE-119, proved to be estrogenic (Meerts et al. 2001). In addition, several BDE congeners found in DE-71 were mildly antiestrogenic in the same assay. Likewise, Hamers et al. (2006) observed weak estrogen activity by several low-brominated BDEs, weak antiestrogenic activity for tetra- and hepta-brominated BDEs and 60H-BDE-47, and neither activity for the DE-71 mixture.

Our results show that in the OVX mouse sc DE-71 produced very small estrogenic effects, whereas po DE-71 had no effect. Because DE-71 had estrogenic effects in cell culture, the lack of effect in po-treated mice suggests rapid clearance via liver metabolism. However, when we analyzed plasma of mice that had been treated with DE-71 for 34 days, we found similar amounts of parent compounds and metabolites in the blood of either sc- or po-treated animals, with the exception of BDE-153, which was 5 times higher in the po group (Qiu et al. 2007). Furthermore, the concentration of total DE-71 congeners was approximately 1,000–2,000 ng/mL, similar to what would be achieved with 2–4 µM treatment in culture, where 1 µM was minimally effective and 5 µM showed an effect that was approximately 50% of the maximal E\textsubscript{2}-induced effect. Staskal et al. (2005) found that nonmetabolized BDE-47 is rapidly cleared in the mouse. This process is mediated by urinary proteins and may be saturated after repeated exposure (Diliberto et al. 2007; Staskal et al. 2006). Because the animals were treated long-term with a high dose of DE-71, it is possible that the concentrations we found in blood represent a steady-state level reached when urinary clearance is at its maximum capacity. Although this may explain why the po- and sc-treated animals had similar blood concentrations of most PBDEs, it does not explain why the sc route produced estrogenic effects while the po route did not. Another explanation is that more PBDE congeners may reach estrogen targets when administered sc rather than po. It is possible that sc-administered PBDEs reached higher levels in peripheral tissue than liver compared with po-administered PBDEs by avoiding the high activity of conjugating (phase II) enzymes in the liver and gut (Cassidy and Houston 1984; Li et al. 2004). It is also possible that PBDEs are activated by CYP in peripheral tissues such as adipose or at the estrogen-target tissues (Shimada et al. 2003, Yoshinari et al. 2006). Others have also seen higher efficacy of sc dosing over po dosing of estrogens, as is the case of the xenoestrogen bisphenol A (Berger et al. 2007) and steroidal estrogen (Savvas et al. 1992).

The MCF-7 cell proliferation assay used here to test DE-71 is probably more sensitive than the reporter gene expression system used by others (Hamers et al. 2006; Legler et al. 1999) due to the longer time of incubation with the chemical (10 days vs. 24 hr), thereby allowing accumulation of both the PBDE congeners inside the cell [increased intracellular concentration (Mundy et al. 2004)] and the estrogenic effect (cell growth). Alternatively, MCF-7 cells are known to express CYP enzymes (Barber et al. 2006; Peters et al. 2004), and it may be that during the 10-day incubation they metabolically convert BDE congeners to more active hydroxylated forms.

Other researchers have shown that both BDE-99 and DE-71 interfere with rodent sexual development after prenatal exposure (Ceccatelli et al. 2006; Kuriyama et al. 2005; Lilienthal et al. 2006), but specific hormonal activity involved was not demonstrated. As noted by Ceccatelli et al. (2006), PBDE remains in the offspring for months after birth, making it impossible to determine if the observed increase in expression of estrogen target genes during adulthood was due to developmental defects or adult hormone-like effects (or a combination of both). Although such research is suitable to assess the sensitivity to developmental effects, it does not define these effects as estrogenic. The pubertal development protocol used by Stoker et al. (2004) and the adult gonadectomized rodent model used here are more suitable to assess estrogenic activity by looking at well-known responses to estrogen after chemical challenge. Classic estrogenic responses such as increased uterine weight, UEH, and VET in the adult OVX mouse are a strong indication of the involvement of estrogen-signaling pathways and are standard methods to assess estrogenicity of a chemical (Clode et al. 2006).

We found differences in responses between mouse strains: The uterine and vaginal epithelium seems to be more sensitive to the effects of DE-71 in BALB/c mice than in C57BL/6 mice. Others have shown that C57BL/6 mice exhibit higher sensitivity to estrogen compared with other strains (Silberberg and Silberberg 1951; Spearow et al. 1999, 2001), but BALB/c mice were not included in those comparisons.

**Figure 4.** Effects of DE-71 on liver weight of mice were treated for either 3 (A) or 34 (B, C) days with DE-71 alone or with E\textsubscript{2} and DE-71. Controls for the 34-day time point were treated po with vehicle. Values shown are mean ± SE (n = 5–10 mice per sc group; n = 4–5 mice per po group).

*p < 0.05, **p < 0.01, and ***p < 0.001 vs. vehicle.

**Figure 5.** Effects of DE-71 on liver microsomal CYP activity in BALB/c mice treated for 34 days with DE-71 alone or with EB plus DE-71. (A) PROD (CYP2B) activity. (B) EROD (CYP1A) activity. Values shown are mean ± SE. n = 8 mice per group.

*p < 0.05, and **p < 0.01 vs. vehicle.
Although our findings may illustrate a real difference in estrogen sensitivity between the BALB/c and C57BL/6 strains, an alternative explanation is that between-assay variability makes it difficult to measure small estrogenic effects (Ashby et al. 2004; Thiigjen et al. 2002; Tinwell et al. 2000).

DE-71 increased liver weight in both BALB/c and C57BL/6 mice treated for 34 days, an effect shown previously in rats (Stoker et al. 2004; Zhou et al. 2001, 2002) and mink (Martin et al. 2007). The fact that a similar increase in liver weight occurred in ER−αKO mice indicates that this is not dependent on ER-α signaling. An increase in liver weight may occur in parallel with histologic changes such as hepatocytomegaly, aci
dodiphilic cytoplasm, binucleated hepatocytes, and eosinophilic bodies (Hardy et al. 2002; Son et al. 2007). Although we did not analyze the tissue histologically, others have shown that oral administration of DE-71 to rats for up to 90 days resulted in increased liver weights, with concomitant histologic changes (International Programme on Chemical Safety 1994). In BALB/c mice treated for 3 days, DE-71 increased liver weight in a dose-dependent manner only when administered po. Interestingly, EB potentiated the DE-71–induced increase in liver weight in BALB/c but not in C57BL/6 mice. Previous studies have shown that an estrogen-induced increase in liver weight in mice is strain dependent (Buchanan et al. 2002; Duffel et al. 1981; Goodin et al. 2002; Wang et al. 2004).

Because DE-71 treatment increased liver weight while at the same time diminishing the action of administered EB on the uterine epithelium (34-day treated mice only), it is possible that DE-71 can alter liver metabolic pathways that regulate systemic estrogen activity. A major pathway responsible for E2 deacti
vation is catalyzed by CYP enzymes, and of those CYP1A have the highest activity for E2 hydroxylation (Lee et al. 2003). A less active enzyme, CYP2B, also may be involved in E2 hydroxylation (Acevedo et al. 2005). Induction of hepatic EROD (CYP1A activity) and PROD (CYP2B activity) has been shown in rats (Stoker et al. 2004; Zhou et al. 2001, 2002) and mink (Martin et al. 2007) treated with DE-71 and mice treated with Bromkal 70-5 DE, a PBDE mixture with congener composition similar to DE-71 (Lundgren et al. 2007). In the present study, EROD (CYP1A) was increased by DE-71 but only when administered orally. PROD (CYP2B) was increased by DE-71 regardless of the route of administration, but the increase occurred to a much greater extent when the po route was used. Because the small antiestrogenic effect observed in the uterine epithelium occurred only when DE-71 was administered sc, it does not appear that this effect can be attributed to altered estrogen metabolism through increased CYP1A or CYP2B.

Individual PBDE congeners are known to induce expression of CYP2B, but the induc
tion of CYP1A activity by PBDEs is most likely due to contamination with polychromi
nated dibenzo-p-furans (Hanari et al. 2006; Kuiper et al. 2006), which are known to induce aryl hydrocarbon receptor signaling in mammals (Olsman et al. 2007). Sanders et al. (2005) found that both DE-71 and its three main component congeners (BDEs 47, 99, and 153) increased CYP2B gene expression in rats, but that only DE-71 and not the individ
ual congeners strongly up-regulated CYP1A, thus suggesting that the CYP1A increase was due to furan contamination of the DE-71 mixture. Because furans do not induce CYP2B, PROD induction is most likely mediated by PBDE activation of the constitutive androstane receptor, CAR (reviewed by Yamada et al. 2006).

In summary, the PBDE mixture DE-71 behaves as a weak estrogen in both MCF-7 breast cancer cell proliferation and the overactiv
ized adult mouse models. The anti
estrogenic activity may be due to an interaction with ERs, not to a metabolic depletion of coadministered estrogens. In animal studies, treatment route and duration determined whether DE-71 was estrogenic or not. BALB/c mice are more susceptible to DE-71 effects in estrogen target tissues and in liver than C57BL/6 mice. DE-71 also increased liver weight in both mouse strains tested, and this effect was not dependent on ER-α. It still remains to be seen if the above listed effects of DE-71 are due to the original BDE congeners or to their hydroxylated metabolites.

**REFERENCES**

Acevedo R, Parnell PG, Villanueva H, Chapman LM, Gimenez T, Gray SL, et al. 2005. The contribution of hepatic steroid metabolism to serum estradiol and estrone concentrations in nonymphal treated MMTVneu mice and its potential effects on breast cancer incidence and latency. J Appl Toxicol 25(5):391–393.

ATSDR. 2004. Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers (PBBS and PBDEs). Atlanta, GA:Agency for Toxic Substances and Disease Registry. Available: http://www.atsdr.cdc.gov/toxprofiles/tp66.html [accessed 19 December 2007].

Ashby J, Tinwell H, Oudem J, Lefevre P. 2004. Natural variability and Female Reproductive Systems (Naz RK, ed). Boca

Clode SA. 2006. Assessment of in vitro models for endocrine disruption. Best Pract Res Clin Endocrinol Metab 20(1):23–43.

Dango MC, Ziegler DM. 2001. Changes in dimethyl
aminonitrosamine-induced gene expression of mouse liver and kidney induced by steroid sex hormones. Mol Cell Biochem 221:1–8.

Evans JS, Varney RF, Koch FC. 1941. The mouse uterine wet weight method for the assay of estrogens. Endocrinology 28:747–753.

Goodin MG, Fertuck KC, Zacheckowski TR, Rosengren RJ. 2002. Estrogen receptor-mediated actions of polybrominated cene-chins in vivo and in vitro. Toxicol Sci 68(2):354–361.

Gordon MN, Osterby HH, May PC, Finch CE. 1998. Effective oral administration of 17-β-estradiol to female C57BL/6J mice through the drinking water. Biol Reprod 59(3):1088–1095.

Great Lakes Chemical Corp. 2006. Material Safety Data Sheet, Great Lakes DE-71™, West Lafayette, IN:Great Lakes Chemical Corporation. Available: http://www.el.greatlakes.com/common/msdspdf/00043.pdf [accessed 3 March 2008].

Hahnle IT, Twaddle E, 1971. Estrogen receptors in human breast cancer. I. Methodology and characterization of receptors. Steroids 16(6):653–680.

Hamers T, Kamstra JH, Sonneveld E, Murk AJ, Kester MH, Andersson PL, et al. 2006. In vitro profiling of the endocrine-disrupting potencies of brominated flame retardants. Toxicol Sci 92(1):157–173.

Hanari N, Kanno K, Miyake Y, Okazawa T, Kodavanti PR, Baldus KM, et al. 2005. Occurrence of polybrominated biphenyls, polybrominated dibenzofurans, and poly
brominated dibenzo[ghi]perfluorocyclobutanes as impurities in commercial polybrominated diphenyl ether mixtures. Environ Sci Technol 40(9):4400–4405.

Hardy ML, Margulich D, Ackerman L, Smith RL. 2002. The sub
chronal oral toxicity of ethane, 1,2-bis(pentabromophenyl) (Saytex 8010) in rats. Int J Toxicol 21(3):195–170.

Hatakeyama M, Zou E, Matsuura F, 2002. Comparison of the characteristic of estrogenic action patterns of beta-HCH and heregulin beta1 in MCF-7 human breast cancer cells. J Biochem Mol Toxicol 16(5):209–219.

Hiles RA. 2004. Polybrominated diphenyl ethers in the environ
ment and in people: a meta-analysis of concentrations. Environ Sci Technol 35(8):945–956.

Hooper K, McDonald TA. 2000. The PBDEs: an emerging envi
ronmental challenge and another reason for breast milk monitoring programs. Environ Health Perspect 108:387–392.

International Programme on Chemical Safety. 1994. Brominated Diphenyl Ethers, Environmental Health Criteria 162. Geneva:World Health Organization.

Kuiper RV, Murk AJ, Leonardes P, Grimes GC, van den Berg M, Vos JG. 2001. In vivo and in vitro Ah-receptor activation by commercial and fractionated pentabromodipheny]ether using zebrafish (Danio rerio) and the Dd-Callux assay. Aquat Toxicol 79(4):366–375.

Kuriyama SN, Talsness CE, Grote K, Chahoud I. 2005. Developmental exposure to low-dose PBDE-99: effects on male fertility and neurobehavior in rat offspring. Environ Health Perspect 113:149–154.

Law RJ, Allchin CR, de Boer J, Covaci A, Herxen D, Lepom P, et al. 2000. The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands. Toxicol Sci 54(1):138–153.
Polybrominated biphenyl and dibenzo-p-dioxin fat storage: analysis, toxicity, and environmental occurrence. Rev Environ Contam Toxicol 141:1–26.
Qiu X, Mercado-Feliciano M, Bigsby RM, Hites RA. 2007. Measurement of polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a commercial pentabromodiphenyl ether mixture. Environ Health Perspect 115:1084–1097.
Sanders JM, Burka LT, Smith CS, Black W, James R, Cunningham ML. 2005. Differential expression of CYP1A2, 2B, and 23A genes in the F344 rat following exposure to a polybrominated diphenyl ether mixture or individual components. Toxicol Sci 84(1):127–133.
Savvas M, Studd JW, Norman S, Heath AT, Garnett T, Fogelman I. 1992. Increase in bone mass after one year of percutaneous oestradiol and testosterone implants in post-menopausal women who have previously received long-term oral oestrogens. Br J Obstet Gynaecol 99(9):757–760.
Shimada T, Sugie A, Shindo M, Nakajima T, Azuma E, Hashimoto M, et al. 2003. Tissue-specific induction of cytochromes P450 1A1 and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in engineered CD8 T cells and mouse of arylichlorohydrin receptor gene. Toxicol Appl Pharmacol 187(1):1–10.
Siddiqui MA, Laessig RH, Reed KD. 2003. Polybrominated diphenyl ethers (PBDEs): new pollutants-old diseases. Clin Med Res 1(4):281–290.
Silberberg M, Silberberg R. 1991. Susceptibility to estrogen of breast, vagina, and endometrium of various strains of mice. Proc Soc Exp Biol Med 199(1):161–164.
Son HY, Kim SH, Shin HI, Bae HI, Yang JH. 2007. Perfluorooctanoic acid-induced hepatic toxicity following 21-day oral exposure in mice. Arch Toxicol doi:10.1007/s00204-007-0246-x (Online 14 September 2007).
Soto AM, Sonnenschein C, Chung XL, Fernandez MF, Olea N, Serrano FD. 1995. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 103(suppl 2):113–122.
Spearow JL, Doemney P, Sera R, Leffler R, Barkley M. 1989. Genetic variation in susceptibility to endocrine disruption by estrogen in mice. Science 240(4859):1295–1298.
Staskal DF, Diliberto JJ, Birnbaum LS. 2006. Impact of repeated exposure on the toxicokinetics of BDE 47 in mice. Toxicol Sci 92(2):380–385.
Staskal DF, Diliberto JJ, Devito MJ, Birnbaum LS. 2005. Inhibition of human and rat CYP1A2 by TCDD and dioxin-like chemicals. Toxicol Sci 82(2):225–231.
Steinmetz R, Young PC, Caperell-Grant A, Gize EA, Madhukar BV, Staskal DF, Diliberto JJ, Devito MJ, Birnbaum LS. 2006. Environmentally relevant xenobiotics: analysis, toxicity, and environmental occurrence. Rev Environ Contam Toxicol 141:1–26.
Suzuki A, Enari M, Abe Y, Ohta Y, Iguchi T. 1996. Effect of ovariectomy on histological change and protein expression in female mouse reproductive tract. In Vivo 10(1):103–110.
Thijen GE, Haseman JK, Saunders H, Locklear J, Caviness G, Grant M, et al. 2002. Dietary factors affecting uterine weights of immature CD-1 mice used in uterotrophic bioassays. Cancer Detect Prev 26(5):381–393.
Timwell H, Joiner R, Patel I, Soames A, Foster J, Ashby J. 2000. Uterotrophic activity of bisphenol A in the immature mouse. Regul Toxicol Pharmacol. 32(1):118–126.
Ulrich EM, Caperell-Grant A, Jung SH, Hites RA, Biggsy RM. 2000. Environmentally relevant xenobiotics: new pollutants-old diseases. Clin Med Res 1(4):281–290.
Welsch CW, Swim EL, McManus MJ, White AC, McGrath CM. 1981. Estrogen induced growth of human breast cancer cells (MCF-7) in athymic nude mice is enhanced by secretions from a transplantable pituitary tumor. Cancer Lett 14(3):309–316.
Yamada H, Ishii Y, Yamamoto M, Oguri K. 2006. Induction of the hepatic cytochrome P450 2B1 subfamily by xenobiotics: research history, evolutionary aspect, relation to tumorigenesis, and mechanism. Curr Drug Metab 7(4):397–409.
Yoshinari K, Okino N, Sato T, Sugatani J, Miwa M. 2006. Induction of the hepatic cytochrome P450 2B1 subfamily by xenobiotics: research history, evolutionary aspect, relation to tumorigenesis, and mechanism. Curr Drug Metab 7(4):397–409.
Zhou T, Taylor MM, DeVito MJ, Crofton KM. 2002. Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. Toxicol Sci 66(1):105–116.