

Affinity for Thyroid Hormone and Estrogen Receptors of Hydroxylated Polybrominated Diphenyl Ethers

Shigeyuki Kitamura,^{*,a,b} Seiji Shinohara,^a Eri Iwase,^a Kazumi Sugihara,^a Naoto Uramaru,^{a,b} Hidenari Shigematsu,^b Nariaki Fujimoto,^a and Shigeru Ohta^a

^aGraduate School of Biomedical Sciences, Hiroshima University, Kasumi 1–2–3, Minami-ku, Hiroshima 734–8553, Japan and ^bNihon Pharmaceutical University, Komuro 10281, Ina-machi, Saitama 362–0806, Japan

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The affinity for thyroid hormone receptor (TR) of polybromodiphenyl ethers (PBDEs) and hydroxylated PBDEs was examined. 4-Hydroxy-2,2',3,4',5-pentabromodiphenyl ether (4-OH-BDE-90) and 3-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (3-OH-BDE-47) markedly inhibited the binding of triiodothyronine (1×10^{-10} M) to TR in the concentration range of 1×10^{-6} – 1×10^{-4} M. 2,3,4,5,6-Pentabromophenol (PBP) also showed an inhibitory effect at 1×10^{-5} – 1×10^{-4} M. However, 2,2',3,4,4',5'-hexabromodiphenyl ether (BDE-138), decabromodiphenyl ether (DBDE), 4-methoxy-2,2',3,4',5-pentabromodiphenyl ether (4-MeO-BDE-90), 4'-hydroxy-2,2',4,5'-tetrabromodiphenyl ether (4'-OH-BDE-49), 4-hydroxy-2,2',3,4'-tetrabromodiphenyl ether (4-OH-BDE-42), 4'-hydroxy-2,2',4-tribromodiphenyl ether (4'-OH-BDE-17), 3'-hydroxy-2,4-dibromodiphenyl ether (3'-OH-BDE-7), 2,4,6-tribromophenol (TBP) and tetrabromohydroquinone (TBHQ) did not show affinity for TR. In contrast, 4'-OH-BDE-17 and 3'-OH-BDE-7 exhibited estrogenic activity in estrogen-responsive reporter assay using MCF-7 cells at the concentration of 1×10^{-5} M. However, adjacent bromo substitution of 3- or 4-hydroxylated PBDEs markedly decreased the estrogenic activity. These results suggest that hydroxylated PBDEs act as thyroid hormone-like agents, as well as estrogens, that a 4- or 3-hydroxyl group in PBDEs is essential for thyroid hormonal and estrogenic activities, and that adjacent dibromo substitution favors thyroid hormonal activity, but not estrogenic activity.

Key words——polybrominated diphenyl ether, thyroid hormonal activity, estrogenic activity, hydroxylated

polybrominated diphenyl ether

INTRODUCTION

Brominated flame retardants are widely used throughout the world in electronic circuit boards and other electronic equipment.^{1–5} Polybrominated diphenyl ethers (PBDEs) and tetrabromobisphenol A (TBBPA) have been detected in environmental samples such as sediments, fish, birds, and also in human breast milk and blood.^{6–14} Exposure to PBDEs can lead to thyroid hormone disturbances and neurotoxicity in wildlife and humans.^{15–19}

It has been reported that some PBDEs and TBBPA have estrogenic, anti-androgenic and anti-progesterone activities *in vitro*.^{19–22} Ceccatelli *et al.* reported that a PBDE, PBDE 99, can cause disruption of estrogenic action *in vivo* in rats.²³ Richardson *et al.* reported that BDE 47 caused disruption of thyroid hormonal action in mice *in vivo*.²⁴ Lilienthal *et al.* also reported an effect of PBDE 99 on sexual development and sexually dimorphic behavior in rats.²⁵ Several PBDEs have been reported to cause thyroid hormone disruption.^{26–28} It has been suggested that hydroxylated PBDEs may disrupt thyroid hormone status, because of their structural similarity to thyroid hormone. Hydroxylated PBDEs may have high binding affinity for the serum thyroid hormone binding protein transthyretin (TTR), thyroid hormone receptor (TR), thereby displacing the natural ligand. However, the exact mechanisms of interference with thyroid hormonal action are not fully understood.

In this report, we tested with the thyroid hormonal and estrogenic activities of PBDEs, including their hydroxylated derivatives, as determined by means of binding assay with TR from the rat pituitary cell line MtT/E-2 and estrogen-

^{**}To whom correspondence should be addressed: Nihon Pharmaceutical University, Komuro 10281, Ina-machi, Saitama 362–0806, Japan. Tel.: +81-48-721-1155; Fax: +81-48-721-6973; E-mail: kitamura@nichiyaku.ac.jp

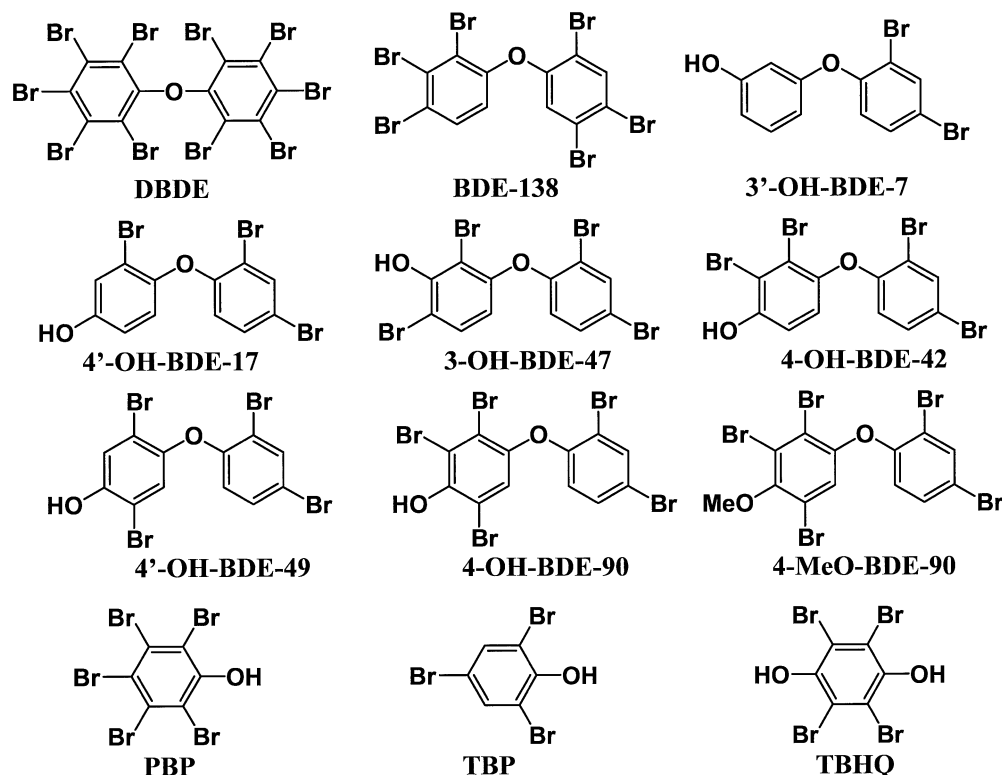


Fig. 1. Structures of PBDEs and Hydroxylated PBDEs Used in this Study

responsive element (ERE)-luciferase reporter assay using MCF-7 cells. As substrates, we used 12 PBDEs and related compounds, 2,2',3,4,4',5'-hexabromodiphenyl ether (BDE-138), decabromodiphenyl ether (DBDE), 4-hydroxy-2,2',3,4'-tetrabromodiphenyl ether (4-OH-BDE-42), 4'-hydroxy-2,2',4-tribromodiphenyl ether (4'-OH-BDE-17), 4-hydroxy-2,2',3,4',5-pentabromodiphenyl ether (4-OH-BDE-90), 3-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (3-OH-BDE-47), 4'-hydroxy-2,2',4,5'-tetrabromodiphenyl ether (4'-OH-BDE-49), 3'-hydroxy-2,4-dibromodiphenyl ether (3'-OH-BDE-7), 4-methoxy-2,2',3,4',5-pentabromodiphenyl ether (4-MeO-BDE-90), 2,3,4,5,6-pentabromophenol (PBP), 2,4,6-tribromophenol (TBP) and tetrabromohydroquinone (TBHQ) (Fig. 1). We found that hydroxylated PBDEs exhibit significant thyroid hormonal activity, as well as estrogenic activity.

MATERIALS AND METHODS

Chemicals — ^{125}I -T3 (3,5,3'- ^{125}I , radiochemical purity > 95%, 28.8 TBq/mmol) was purchased from NEN Life Science Products (Boston, MA,

U.S.A.). Hydroxylated PBDEs and other PBDEs were obtained from Accu Standard (New Haven, CT, U.S.A.), and 17β -estradiol (E2) was from Sigma (St. Louis, MO, U.S.A.). Test compounds were solubilized in dimethyl sulfoxide, and the assay was conducted in 0.1% dimethyl sulfoxide solution. Cells of a human breast cancer cell line, MCF-7, were obtained from the Health Science Research Resources Bank (Osaka, Japan). The rat pituitary cell line, MtT/E-2, was established in our laboratory.²⁹⁾

Competitive Binding Assay to TR — Nuclear extracts of MtT/E were used for the assay, since this cell line expresses large amounts of TR. MtT/E-2 cells were homogenized in 0.32 M sucrose solution containing 3 mM MgCl_2 and 1 mM dithiothreitol, and centrifuged at 700 *g* for 10 min. The pellets were resuspended in 2.4 M sucrose with MgCl_2 and centrifuged at 53000 *g* for 45 min. The resulting nuclear pellets were resuspended in TMDS buffer (2 mM Tris-HCl, 3 mM MgCl_2 , 1 mM dithiothreitol, 0.32 M sucrose, pH 7.4). Various concentrations of test chemicals and 3 nM ^{125}I -T3 were incubated in 0.2 ml of the nuclear suspension at 37°C for 40 min. After incubation, 0.25 ml of 2% Triton X-100 was added to terminate the reaction, and the mixture

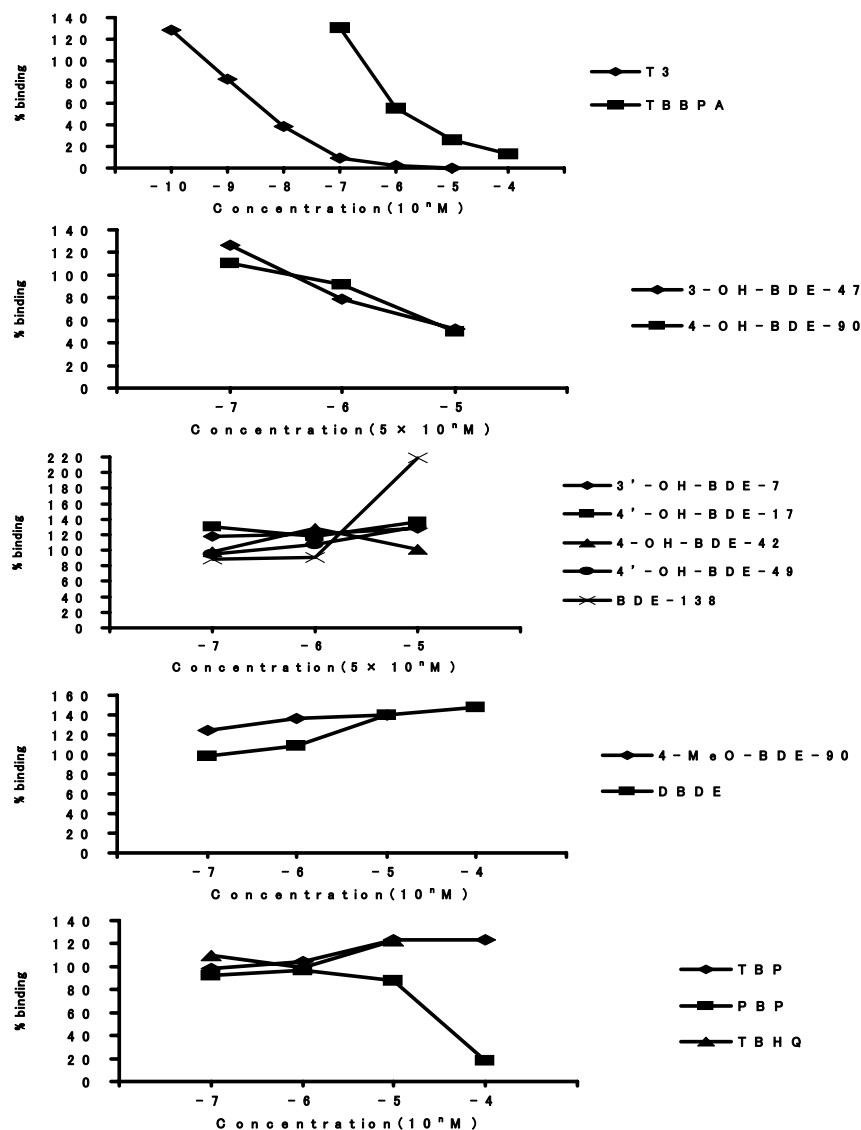


Fig. 2. Binding Assay of Test Compounds to Thyroid Hormone Receptor

Each value represents the mean of triplicate determinations. Activity was expressed relative to the control with no added test compound. T3; L-3,5,3'-triiodothyronine, TBBPA; tetrabromobisphenol A, 3-OH-BDE-47; 3-hydroxy-2,2',4,4'-tetrabromodiphenyl ether, 4-OH-BDE-90; 4-hydroxy-2,2',3,4',5-pentabromodiphenyl ether, 3'-OH-BDE-7; 3'-hydroxy-2,4-dibromodiphenyl ether, 4'-OH-BDE-17; 4'-hydroxy-2,2',4-tribromodiphenyl ether, 4-OH-BDE-42; 4-hydroxy-2,2',3,4'-tetrabromodiphenyl ether, 4'-OH-BDE-49; 4'-hydroxy-2,2',4,5'-tetrabromodiphenyl ether, BDE-138; 2,2',3,4,4',5'-hexabromodiphenyl ether, 4-MeO-BDE-90; 4-methoxy-2,2',3,4',5-pentabromodiphenyl ether, DBDE; decabromodiphenyl ether, TBP; 2,4,6-tribromophenol, PBP; 2,3,4,5,6-pentabromophenol, TBHQ; tetrabromohydroquinone.

was centrifuged at 1000 *g* for 10 min. The pellets were washed 2 times with 1 ml of TMDS buffer and the supernatant was removed. Radioactivity of the pellets was counted with a gamma counter (Wallac Wizard 1480, Perkin Elmer Life Sciences, Boston, MA, U.S.A.).

Assay of Estrogenic Activity of Hydroxylated PBDEs and Related Compounds—ERE-luciferase reporter assay using MCF-7 cells was performed according to the previously reported method.³⁰⁾

RESULTS

Competitive Binding Assay for TR of Hydroxylated PBDEs and Related Compounds

The inhibitory effects of PBDEs and hydroxylated PBDEs on binding of T3 to TR were examined. T3 competitively inhibited the binding of ¹²⁵I-T3 (1×10^{-10} M) to TR in the range of 1×10^{-9} – 1×10^{-6} M, and TBBPA also showed an inhibitory effect at 10^{-6} – 10^{-4} M. 4-OH-BDE-90 and 3-OH-BDE-47 also markedly inhibited the binding of

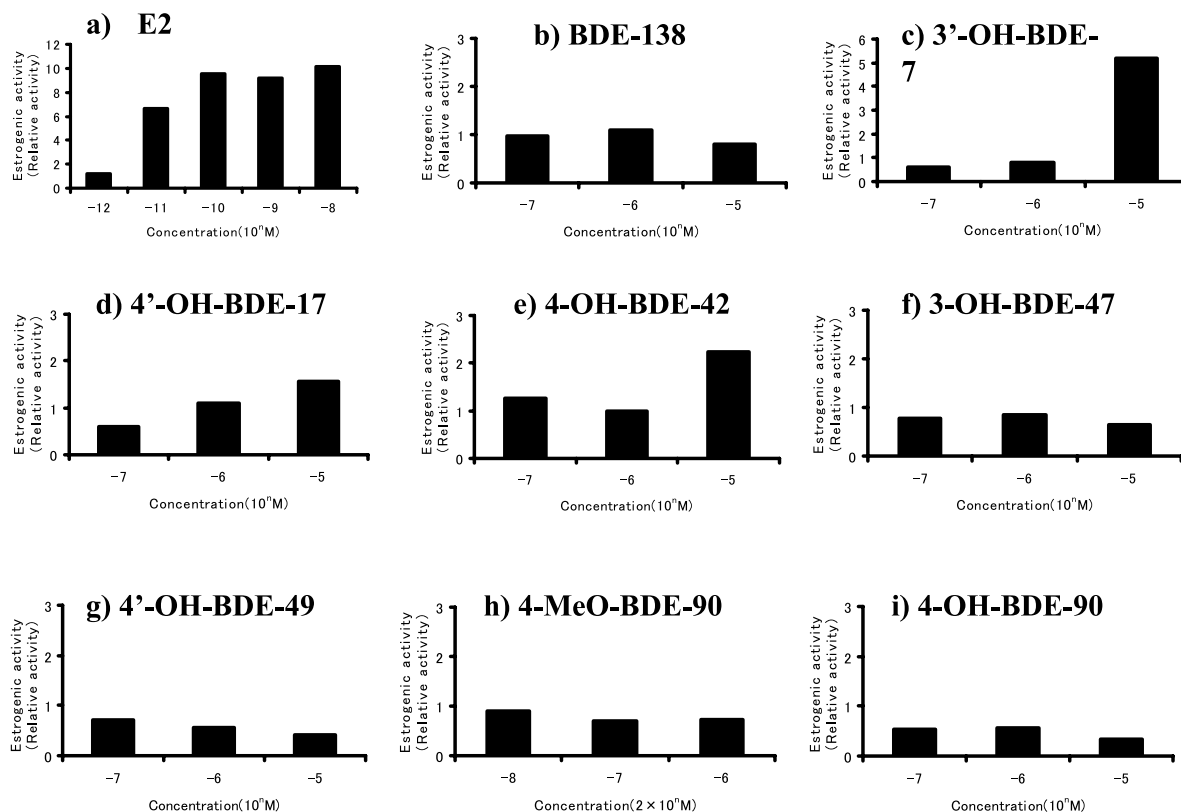


Fig. 3. Estrogenic Activity of PBDEs and Related Compounds in ERE-luciferase Reporter Assay Using MCF-7 Cells

Each value represents the mean of triplicate determinations. Estrogenic activity of PBDEs was expressed as relative activity with respect to the control using MCF-7 cells. E2; 17β -estradiol, BDE-138; 2,2',3,4,4',5'-hexabromodiphenyl ether, 3'-OH-BDE-7; 3'-hydroxy-2,4-dibromodiphenyl ether, 4'-OH-BDE-17; 4'-hydroxy-2,2',4-tribromodiphenyl ether, 4-OH-BDE-42; 4-hydroxy-2,2',3,4'-tetrabromodiphenyl ether, 3-OH-BDE-47; 3-hydroxy-2,2',4,4'-tetrabromodiphenyl ether, 4'-OH-BDE-49; 4'-hydroxy-2,2',4,5'-tetrabromodiphenyl ether, 4-MeO-BDE-90; 4-methoxy-2,2',3,4',5-pentabromodiphenyl ether, 4-OH-BDE-90; 4-hydroxy-2,2',3,4',5-pentabromodiphenyl ether.

^{125}I -T3 to the receptor in the concentration range of 1×10^{-6} – 1×10^{-4} M. PBP showed an inhibitory effect at 1×10^{-5} – 1×10^{-4} M. BDE-138, DBDE, 4-MeO-BDE-90, 4'-OH-BDE-49, 4-OH-BDE-42, 4'-OH-BDE-17, 3'-OH-BDE-7, TBP and TBHQ showed little or no affinity. Elevated binding affinity was observed for hydroxylated PBDEs with bromine substitution at both adjacent positions to the hydroxyl group. 4-Hydroxy-PBDEs brominated at the 3- or 5-position, but not both, showed little activity. Hydroxylated PBDEs with no adjacent bromine substitution of the phenyl ring, and 4-methoxy-PBDEs also showed little affinity (Fig. 2).

Estrogenic Activity of PBDEs and Hydroxylated PBDEs

4'-OH-BDE-17 and 3'-OH-BDE-7 exhibited estrogenic activity in estrogen-responsive reporter assay using MCF-7 cells in the concentration range of 1×10^{-6} – 1×10^{-5} M. However, no estrogenic activity of other 4-hydroxy-PBDEs or PBDEs was

observed. These experiments indicate that a hydroxyl group of PBDEs is essential for estrogenic activity, but 3- or 4-hydroxyl PBDEs with ortho-bromine substituents show decreased estrogenic activity (Fig. 3).

DISCUSSION

In this study, the thyroid hormonal potency of PBDEs was examined, in view of their structural resemblance to the thyroid hormones. We found that hydroxylated PBDEs show thyroid hormone-disrupting activity through interaction with TR, though PBDEs do not. Recently, thyroid hormone-disrupting action of some PBDEs and hydroxy-PBDEs has been discussed.³¹⁾ Meerts *et al.* reported that some PBDEs exhibited binding capability to TTR after metabolic conversion by rat liver microsomes.³²⁾ They suggested that the hydroxylated PBDEs thus formed exhibited the binding ac-

tivity with TTR. Hallgren and Danerud also suggested that hydroxylated metabolites of 2,2',4,4'-tetrabromodiphenyl ether bind TTR in rats *in vivo*.¹⁵⁾ Schriks *et al.* reported that synthetic T2, T3 and T4-like PBDEs show thyroid hormone-like activity.³³⁾ Dietrich *et al.* reported that some brominated thyroxine analogues have thyroid hormonal activity.³⁴⁾ Hamers *et al.* reported that some PBDEs disrupt the endocrine system by binding to TTR, and exhibit progesterone receptor (PR)- and androgen receptor (AR)-antagonistic effects in the presence of T3.¹⁹⁾ We present here direct evidence that some hydroxylated PBDEs bind to TR.

We found that PBDEs substituted with two bromo atoms adjacent to the hydroxyl group on an aromatic ring show thyroid hormonal activity, though other PBDEs do not. A 3- or 4-hydroxyl group and two adjacent bromo substituents on the phenyl group seem to be essential structural factors for binding to TR. In this respect, the compounds apparently resemble T4, rather than T3. However, the atomic size of bromine is much smaller than that of iodine. In view of the affinity of hydroxylated PBDEs for TR, two adjacent bromo substituents may be favorable for binding to TR. We also recognized a similar structural requirement for TR affinity in a brominated bisphenol A, TBBPA, and also chlorinated and methylated bisphenol A.²⁰⁾ 3,5-Substituents of bisphenol A are also necessary for thyroid hormonal activity. Another brominated phenyl ring substituted at the 1-position of the phenyl ring bearing the 3- or 4-hydroxyl group also seems to be necessary for high binding affinity for TR, because TBHQ and TBP showed weaker activity. Hydroxy-polychlorinated biphenyls (PCBs) were also reported to show both estrogenic and thyroid hormone-disrupting activities.^{35–38)} Iwasaki *et al.* reported that 4-hydroxy-2',3,3',4',5'-pentachlorobiphenyl acts as an antagonist by suppressing the interaction of TR and a coactivator.³⁹⁾ We also reported that a 4-hydroxyl group and adjacent 3,5-dichloro substituents on the phenyl group seem to be essential structural factors for binding to TR, in agreement with the present findings.³⁵⁾ In contrast, we found that 4-OH-2',4',6'-trichlorobiphenyl, a 4-hydroxy-PCB without 3,5-chlorine atoms, is estrogenic in an estrogen-responsive reporter test using human breast cancer cell line MCF-7. However, 4-hydroxy-3,5-dichlorinated biphenyl exhibited little estrogenic activity.³⁵⁾ These results are also consistent with the estrogenic activity of hydroxylated PBDEs found in

this study.

There are other mechanisms through which environmental contaminants may interact with the thyroid hormone system. These include direct toxicity at the thyroid gland, which can lead to decreased synthesis of thyroid hormones, disturbance of thyroid hormone metabolism, and interaction with thyroid hormone transport proteins.^{40–44)} Richardson *et al.* demonstrated the induction of glucuronidation and thyroid hormone transporter as mechanisms leading to a decreased level of thyroid hormone after administration of BDE 47 to mice.²⁴⁾ PBDE-99 reduced the thyroxin level in rat dams after a single gestational exposure, and also reduced hepatic enzyme activity.⁴⁵⁾ Hydroxylated PBDEs bind to human TTR and thyroid-binding globulin *in vitro*.³²⁾ In the current study, it was shown that hydroxylated PBDEs interact with TR. These results suggest that hydroxylated PBDEs have the potential to disrupt thyroid hormonal activity *in vivo* by interaction with TR, besides binding with TTR. Further work is necessary to assess the *in vivo* endocrine-disrupting action of hydroxylated PBDEs, taking into account the other thyroid hormone-disrupting actions of these compounds. Thyroid hormonal and estrogenic activities of hydroxylated PBDEs observed *in vitro* may reflect endocrinal toxicity *in vivo*. We demonstrated that amphibian metamorphosis is suppressed by TBBPA and related compounds.^{46, 47)}

In conclusion, the structural requirements of hydroxylated PBDEs for thyroid hormonal activity are a 3- or 4-hydroxyl group and two adjacent bromine substitutions adjacent to the hydroxyl group. The requirement for estrogenic activity is a 3- or 4-hydroxyl group, but adjacent bromine substitution of hydroxy-PBDEs reduces the estrogenic activity.

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