

DETERMINATION OF ESTROGEN RECEPTOR β -MEDIATED ESTROGENIC POTENCIES OF HYDROXYLATED PCBs BY A YEAST TWO-HYBRID ASSAY

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Introduction

Several environmental phenolic chemicals such as Nonylphenol and Bisphenol A (BPA) have been previously shown to possess estrogenic properties. In the previous paper, we have investigated the estrogenic activity of a series of hydroxylated PCBs (OH-PCBs) by a yeast two-hybrid assay (estrogen receptor α (ER α) -TIF2), in which the expression of estrogenic activity is based on the interaction of chemicals with ER α , and demonstrated that 4'-OH-CB30 and 4'-OH-CB61 are more estrogenic than BPA, one of the environmental estrogens^{1,2}. We have showed that one chlorine substitution adjacent to 4-OH at 3- or 5-position significantly reduces the ER α -mediated estrogenic activity of 4-OH-PCBs^{1,2}. Thus, 4'-OH-CB25 and 4-OH-CB56 showed a very weak estrogenicity. We have also showed that 4-OH-PCBs with two chlorine substitutions adjacent to 4-OH at 3- and 5-position such as 4'-OH-CB79 (hydroxylated metabolite of CB77) and persistent 4-OH-PCBs retained in human blood (4-OH-CB107, 4-OH-CB146 and 4-OH-CB187) have no ER α -mediated estrogenic activity^{1,2}.

ER is known to have two subtypes, namely ER α and ER β and it is reported that ligand, some agonist and antagonist have a different binding affinity for ER α and ER β . However, there is limited information on ER β -mediated endocrine disrupting potency.

In this study, we examined the ER β -mediated estrogenic activity of a series of OH-PCBs, including environmentally relevant 4-OH-PCBs by a yeast two-hybrid assay (ER β -TIF2).

Methods and Materials

All OH-PCBs used in this study were synthesized as reported previously except for 4-OH-CB39³. 4-OH-CB39 was purchased from AccusStandard. The chemical structures tested in the yeast two-hybrid assay are presented in Figure 1.

The ER β -mediated estrogenic activity of OH-PCBs was examined using a yeast two-hybrid assay system with the ER β and the coactivator, transcriptional intermediary factor 2 (TIF2), as described previously⁴. Briefly, the yeast cells were preincubated overnight at 30°C in SD medium free from tryptophan and leucine. The culture (250 μ l) was then mixed in a small test tube with a DMSO

solution (2.5 μ l) containing test chemicals and incubated for 4 hr at 30°C. After collecting the cells by centrifugation, the cells were digested enzymatically by incubation with 200 μ l of Zymolyase (1 mg/ml) at 37°C for 15 min. The lysate was mixed with 40 μ l of 2-nitrophenyl- β -D-galactoside (4 mg/ml) and reacted at 30 until development of the yellow color before the reaction was stopped by addition of 1 M Na₂CO₃ (100 μ l). β -Galactosidase activity was determined as described previously.

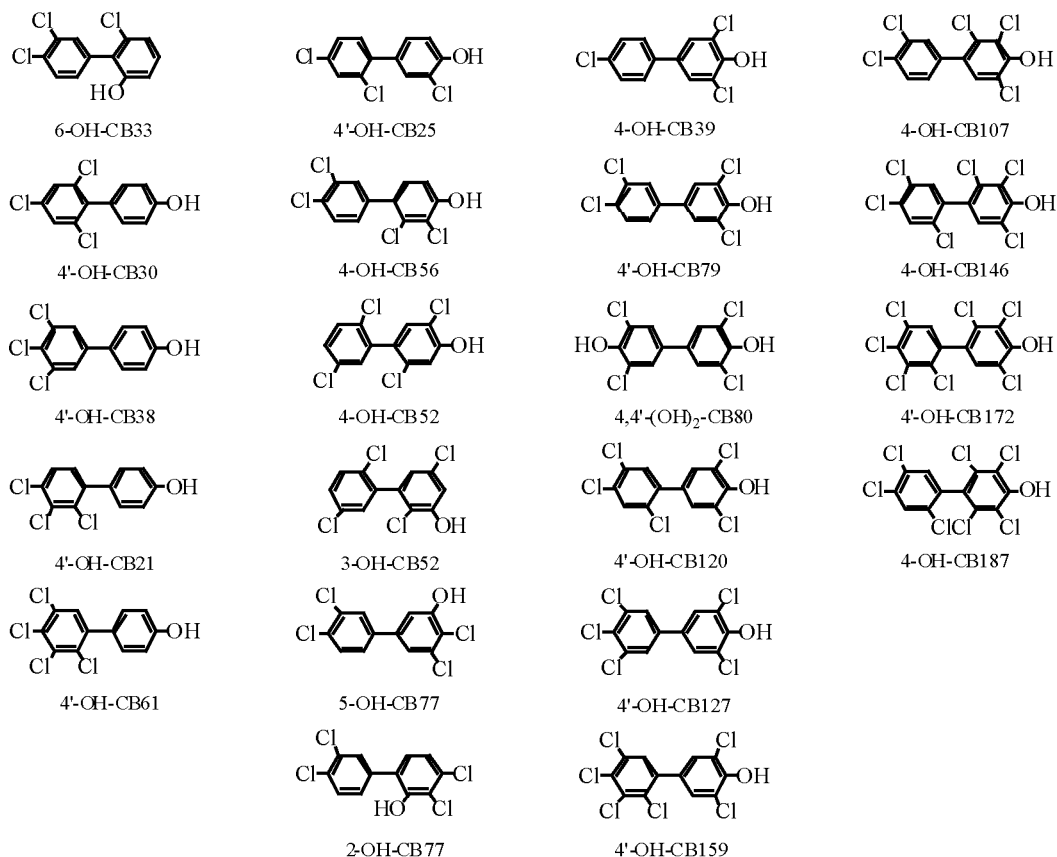


Figure1. Chemical structures of hydroxylated PCBs used in this study.

Results and Discussion

In this yeast two-hybrid assay system (ER β -TIF2), 5×10^{-11} M 17 β -estradiol (E2) caused a slight induction of β -galactosidase activity and this induction increased with E2 concentration (5×10^{-11} M $\sim 1 \times 10^{-7}$ M) as shown in Fig.2. The induced β -galactosidase activity was almost saturated at 1×10^{-8} M of E2.

4'-OH-CB30, 4'-OH-CB61, a mixture of 4'-OH-CB21 and 4'-OH-CB38 (1:1) and 4,4'-(OH)₂-CB80 (dihydroxylated metabolite of CB77) were found to show a significant ER β -mediated activity. The relative estrogenic potencies were evaluated by the concentrations of test compounds showing 10% activity of 5×10^{-10} M E2, REC10 (10% relative effective concentration). The REC10 of E2 was 1.12×10^{-11} M $\sim 2.15 \times 10^{-11}$ M and the relative potencies of test compounds were determined based on each REC10s to that of E2 (Table 1, E2:100). The relative potencies of OH- and (OH)₂-PCBs mentioned above were 1.35, 0.81, 0.19~0.47 and 0.035, respectively and were much higher than those of 4-Nonylphenol (0.012) and BPA (0.002). We have previously reported that these OH- and (OH)₂-PCBs also exhibit a strong ER α -mediated estrogenicity, with the relative potencies within the range of 1.0~0.006^{1,2}. Especially 4'-OH-CB30 was found to be the most potent agonist for both ER α and ER β . It is noteworthy to point out that 4-OH-PCBs with no chlorine substitutions on 4-OH phenyl ring possess high estrogenic activity except for 4,4'-(OH)₂-CB80.

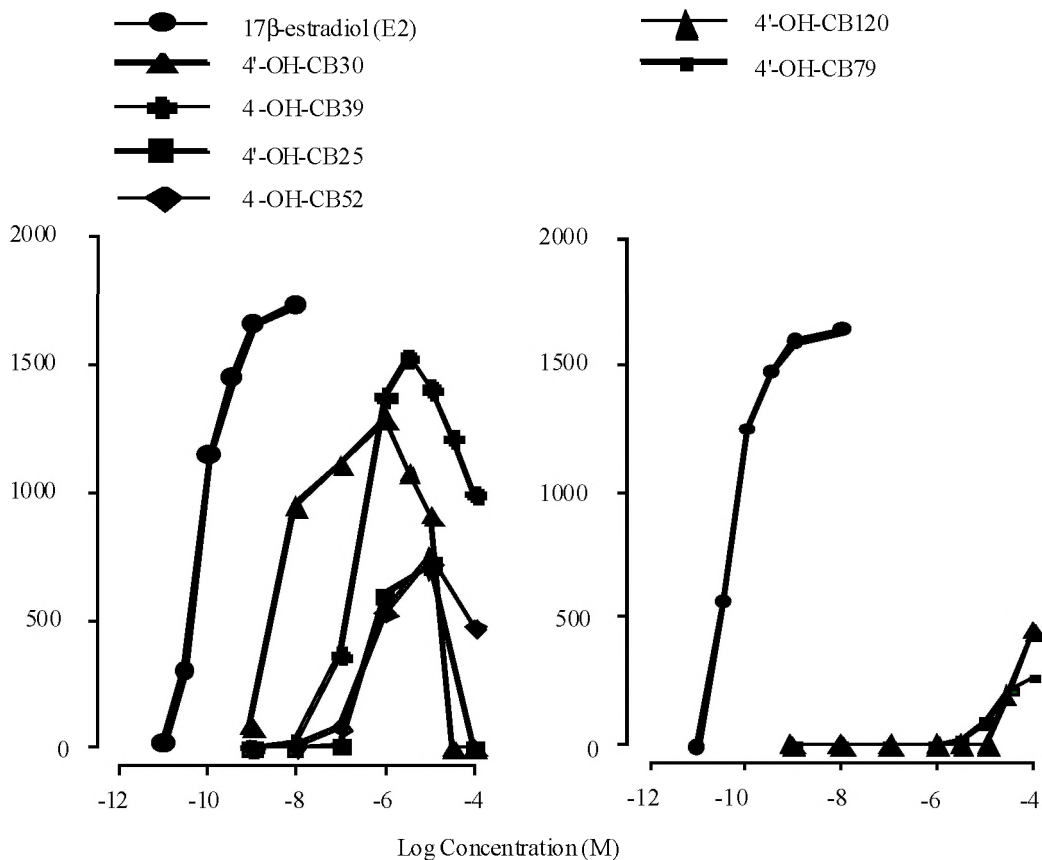


Figure 2. ER β -mediated estrogenic activity of several OH-PCBs in the yeast two-hybrid assay.

Y axis : β -Galactosidase activity unit

4'-OH-CB25 and 4'-OH-CB52 (4-hydroxylated metabolite of CB52) which have one chlorine substitution adjacent to 4-OH at 3- or 5-position (4-OH-3-chloro- type) showed a weak ER β -mediated estrogenic activity, with relative potencies being 0.007 and 0.004~0.007, respectively, although the ER α -mediated estrogenic activity of 4'-OH-CB52 was not detectable.

In the previous study, none of 4'-OH-PCBs with two chlorine substitutions at 3- and 5-position (4'-OH-3,5-dichloro- type) such as 4'-OH-CB39, 4'-OH-CB79 (hydroxylated metabolite of CB77), 4'-OH-CB120, 4'-OH-CB127 (hydroxylated metabolite of CB126) and 4'-OH-CB159 showed the ER α -mediated activity^{1,2}. On the other hand, in the present study, some of 4'-OH-PCBs with 3,5-dichlorosubstitution were found to exhibit ER β -mediated activity. 4'-OH-CB39 was a significant estrogenic with relative potency 0.03~0.05, while 4'-OH-CB79 and 4'-OH-CB120, minor components of 4'-OH-PCBs retained in human blood, showed a weak estrogenic activity, albeit at

concentrations exceeding 10 μ M. The relative potencies were in the range of 0.00003 ~ 0.00005. However, other 4-OH-PCBs with 3,5-dichlorosubstitution, 4'-OH-CB127 (hydroxylated metabolite of CB126) and 4'-OH-CB159, which were also detected in human blood, did not show the ER β -mediated estrogenic activities.

Persistent 4-OH-PCBs (4-OH-2,3,5-trichloro- type) selectively retained in human blood as major components, 4-OH-CB146, 4'-OH-172 and 4-OH-CB187, were found not to be ER β -mediated estrogenic, as well as the results previously obtained with ER α .

Any of 2-OH- or 3-OH-PCBs did not exhibit the ER α -mediated estrogenic activity in the previous study. In this study, most of the 2-OH- and 3-OH-PCBs including 2-OH-CB77 and 5-OH-CB77 (3-hydroxylated metabolite of CB77) also did not show the ER β -inducing activity. However, several 2-OH- and 3-OH-PCBs such as 6-OH-CB33 and 3-OH-CB52 (3-hydroxylated metabolite of CB52) were found to be weakly ER β -mediated estrogenic with relative potencies 0.001 and 0.0003 ~ 0.0007, respectively.

Table 1. ER β -mediated estrogenic potency of hydroxylated PCBs in the yeast two-hybrid assay (ER β -TIF2)

Congener	Relative potency *	Congener	Relative potency *
6 -OH-CB33	0.001	4 -OH-CB52	0.004~0.007
4'-OH-CB30	1.35	3 -OH-CB52	0.0003~0.0007
4'-OH-CB38 + 4'-OH-CB21	0.19~0.47	4 -OH-CB39	0.03~0.05
4'-OH-CB61	0.81	4'-OH-CB79	0.00003
4'-OH-CB25	0.007	4,4'-(OH) ₂ -CB80	0.035
4 -OH-CB56	0.008	4'-OH-CB120	0.00005

* 17 β -estradiol (E2) : 100

In conclusion, a number of OH-PCBs, including several 2-OH- and 3-OH-PCBs, as well as 4-OH-PCBs, showed ER β -mediated estrogenic activity with relative potencies varying 1.35 ~ 0.00003, and the estrogenic activity profile of OH-PCBs with ER β was different from that of ER α . 4'-OH-CB30 is the most potent agonist for both ER α and ER β . Chlorine substitution(s) adjacent to 2-OH-, 3-OH- or 4-OH- significantly reduced the ER β -mediated estrogenic potency. Of the persistent OH-PCBs retained in human blood, only 4'-OH-CB79 and 4'-OH-CB120 exhibited the ER β -mediated estrogenic activity at M concentrations.

References

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