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Table 1
Receptor-binding characteristics of bisphenol A and AF for estrogen receptor α (ERα), ERβ, and estrogen-related receptor γ (ERRγ).

Compounds	Receptor binding potency IC_{50} (nM)[a]		
	ERα	ERβ	ERRγ
17β-estradiol	0.88 ± 0.04	2.17 ± 0.12	NB[b]
4-OHT	2.88 ± 0.15	3.17 ± 0.24	10.3 ± 0.8
bisphenol A	1,030 ± 70	900 ± 70	9.70 ± 0.59
bisphenol AF	53.4 ± 3.1	18.9 ± 0.84	358 ± 3.1
HPTE	59.1 ± 1.5	18.1 ± 1.9	36.4 ± 4.4

[a]IC_{50}, the half maximal inhibitory concentration, was calculated as a measure of the effectiveness of each compound in inhibiting the binding of [^{3}H]17β-estradiol to ERα and ERβ, and [^{3}H]bisphenol A to ERRγ.

[b]NB means "not bound", indicating no significant receptor binding at the 10 μM concentration.

Table 2
Receptor-binding selectivity of bisphenol A and AF for estrogen receptor α (ERα) and β (ERβ) and estrogen-related receptor γ (ERRγ).

Compounds	Receptor binding selectivity			
	ERα vs. ERβ	ERα vs. ERRγ	ERβ vs. ERRγ	Preferred receptor(s)
17β-estradiol	2.47 ERα[a]	(ERα)[b]	(ERβ)[b]	ERα
4-OHT	1.10 ERα	3.58 ERα	3.25 ERβ	ERα~ERβ
bisphenol A	1.14 ERβ	106.18 ERRγ	92.78 ERRγ	ERRγ
bisphenol AF	2.83 ERβ	6.70 ERα	18.94 ERβ	ERβ
HPTE	3.27 ERβ	1.63 ERRγ	2.01 ERβ	ERβ

[a]"2.47 ERα" means that, when the receptor binding selectivity was compared between ERα and ERβ, 17β-estradiol binds to ERα 2.47 times more strongly than to ERβ.

[b](ERα) and (ERβ) mean that, because of inactivity of 17β-estradiol in ERRγ, 17β-estradiol is active exclusively in ERα and ERβ, respectively.

Table 3
Binding affinities of 17β-estradiol, bisphenol A and AF relative to their potencies for stimulating reporter gene activity by ERα and ERβ in HeLa cells.

Compounds	Receptor activation potency EC_{50} (nM) / IC_{50} (nM)	
	ERα	ERβ
17β-estradiol	0.085 (1.0)	0.041 (1.0)
bisphenol A	0.308 (3.6)	0.770 (18.8)
bisphenol AF	1.099 (12.9)	—

Figure Legends

Figure 1. Chemical structures of (A) bisphenol A, (B) bisphenol AF, (C) HPTE, and (D) 17β-estradiol.

Figure 2. Radio-ligand receptor-binding assays of bisphenol AF, bisphenol A, and 17β-estradiol for ERα and ERβ.

(A) Concentration-dependent curves of 17β-estradiol , bisphenol AF, and bisphenol A in the receptor competitive binding assay to measure the ability of the compounds to displace [^{3}H]17β-estradiol in the recombinant human estrogen receptor ERα, and (B) [^{3}H] 17β-estradiol in ERβ. The graphs show representative dose-dependent binding curves, which give the IC_{50} value closest to the mean IC_{50} from at least five independent assays. The IC_{50} values showed a between-experiment coefficient of variation of 5-12%.

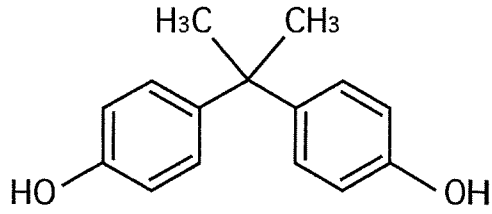
Figure 3. Luciferase-reporter gene assays of bisphenol AF, bisphenol A, and 17β-estradiol for ERα and ERβ.

(A) Concentration-dependent responses of 17β-estradiol, bisphenol AF, and bisphenol A in the luciferase-reporter gene assay for ERα, and (B) for ERβ. Reporter gene (pGL3/3xERE) and either ERα or ERβ expression plasmid (pcDNA3/ERα or pcDNA3/ERβ) were used in HeLa cells. For ERα, bisphenol AF displays full activation in a concentration-dependent manner, while for ERβ it displays extremely weak activity. 17β-Estradiol exhibits very strong activity, with approximately 4.5 times more activity induced at 10^{-14} – 10^{-5} M than at baseline.

Figure 4. Effects of bisphenol AF on the agonist activity of 17β-estradiol in the luciferase-reporter gene assays for ERβ.

(A) Concentration-dependent luciferase-reporter activities of 17β-estradiol are shown by fold activation in the presence and absence of bisphenol AF. 0.1 μM, 1 μM, and 10 μM concentrations of bisphenol AF clearly weaken the agonist activity of 17β-estradiol for ERβ. (B) Concentration-dependent effects of bisphenol AF on the agonist activity of 17β-estradiol. The agonist activity of 10 nM 17β-estradiol was clearly inhibited by bisphenol AF in a dose-dependent manner. Bisphenol AF itself sustained extremely weak activity for ERβ. In all these assays, reporter gene (pGL3/3xERE) and ERβ expression plasmid (pcDNA3/ERβ) were measured in HeLa cells.

A



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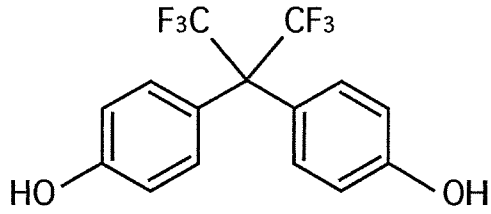

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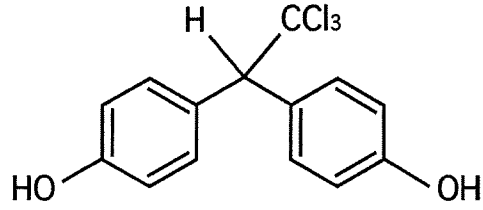

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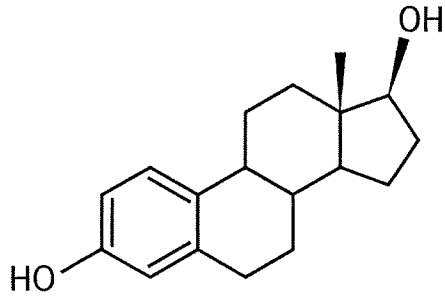

Fig. 1

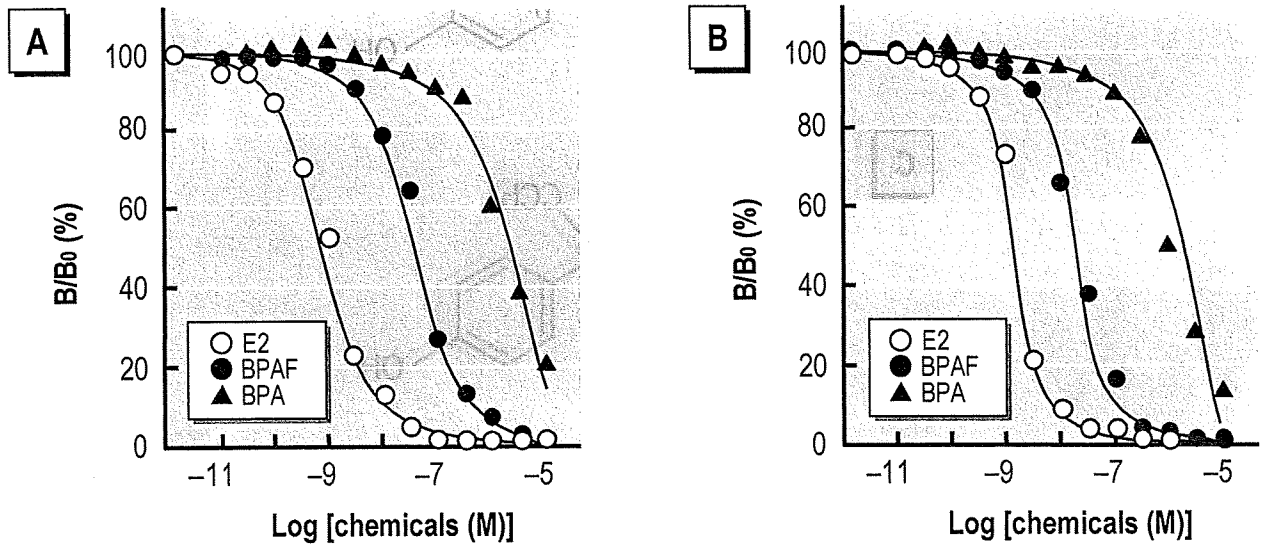

Fig. 2

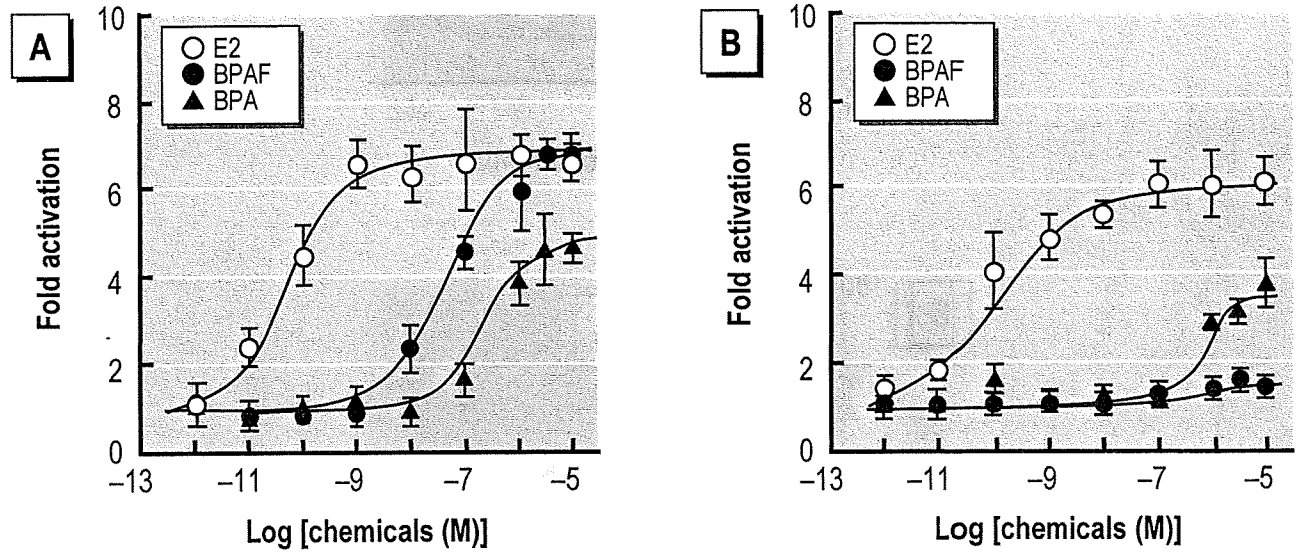
A
B
E2
BPAF
BPA
Fold activation
Log [chemicals (M)]
0
2
4
6
8
10
−13
−11
−9
−7
−5

Fig. 3

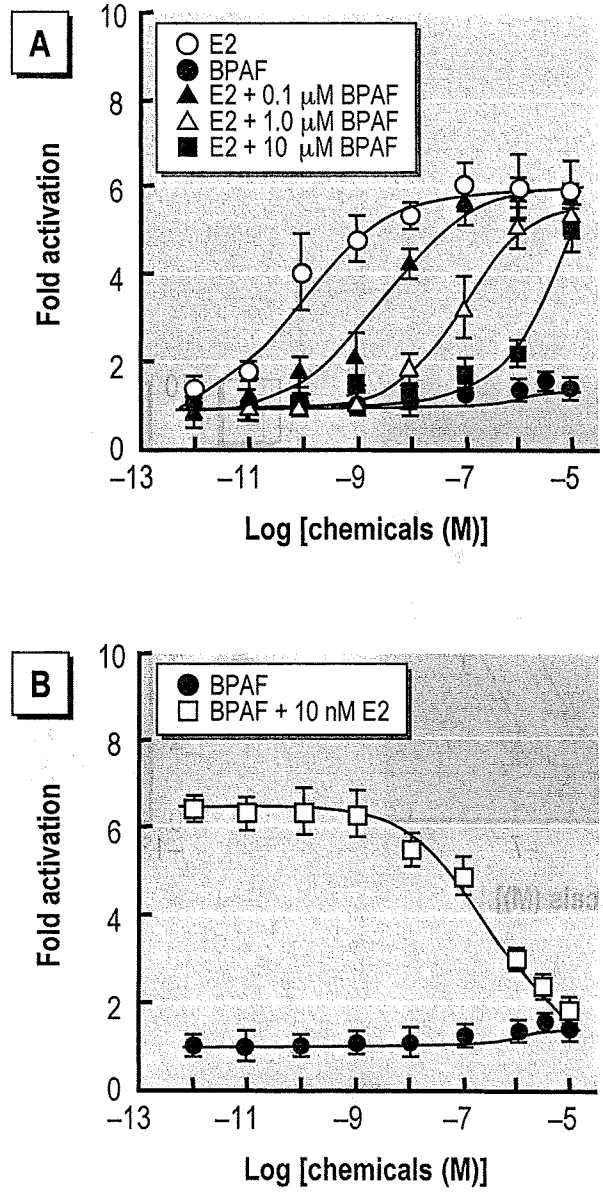
A
E2
BPAF
E2 + 0.1 μM BPAF
E2 + 1.0 μM BPAF
E2 + 10 μM BPAF
Fold activation
Log [chemicals (M)]
B
BPAF
BPAF + 10 nM E2
Fold activation
Log [chemicals (M)]

Fig. 4