DIFFERENTIAL EFFECTS OF POLYBROMINATED DIPHENYL ETHERS AND POLYCHLORINATED BIPHENYLS ON INTRACELLULAR SIGNALING IN RAT NEURONAL CULTURES

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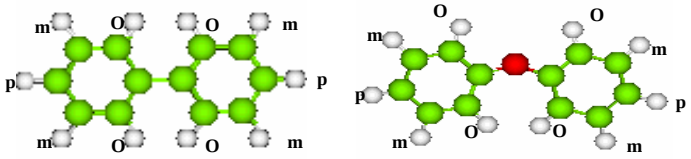
Introduction

Polybrominated diphenyl ethers (PBDEs) are used as additive flame retardants in electrical equipment, plastics, and building materials. Their global production is in the range of 80 million pounds annually[1], and they are becoming ubiquitous contaminants because of high production, lipophilic characteristics and persistence in the environment. PBDEs have similar chemical structure and physicochemical properties to that of other persistent pollutants such as polychlorinated biphenyls (PCBs) (Figure 1) and dichlorodiphenyltrichloroethane (DDT). PBDEs have been detected in human blood, adipose tissue and breast milk. Long-term exposure to these contaminants during development may pose a health risk, especially to children. PBDEs have been increasing in the past 20-30 years, while the presence of other persistent organic pollutants, such as PCBs and dioxins, have decreased in environmental and human samples[2]. If the trends in PBDE levels in human tissues and the environment continue, these chemicals will replace PCBs/DDT as the major environmental persistent organic pollutants over the next 15-30 years. In spite of their widespread occurrence in the environment, only limited information is available on the toxicology of PBDEs. Recent studies showed that PBDE exposure can cause aberrations in spontaneous behavior and reduced learning and memory in mice[3-4]; these effects are similar to those seen after exposure to DDT or PCBs[5], although the mode of action remains unclear.

Previously, we demonstrated that PCBs, which are known to cause neurotoxic effects, affected intracellular signaling pathways including [^{3}H]arachidonic acid ([^{3}H]AA) release, calcium homeostasis, and translocation of protein kinase C (PKC)[6,7]. All these signaling pathways have been associated with learning and memory, and the development of the nervous system[8]. The objectives of the present study were to: (a) test whether a PBDE mixture (DE-71) affected intracellular signaling processes in a similar way to that of PCBs and other organohalogens; and (b) compare the potency and efficacy of PBDEs and PCBs on intracellular signaling.



Biphenyl Ring System **Diphenyl Ether Ring System**

FIG. 1: Structural features of biphenyls and diphenyl ethers

Materials and Methods

Chemicals. All radiolabeled chemicals were purchased from Dupont NEN Corporation (Boston, MA). PBDEs (one mixture, DE-71 and a congener, 2,2',4,4'-tetrabromodiphenylether [PBDE 47]) were a gift from Great Lakes Chemicals. PCBs (one mixture, Aroclor 1254 and a congener, 2,2',4,4'-tetrachlorobiphenyl [PCB 47]) were purchased from AccuStandard (New Haven, CT). PBDEs and PCBs were dissolved in dimethyl sulfoxide (DMSO) and the final concentration in the assay buffer (0.2 to 0.4% v/v) did not significantly affect any of the assays.

Cerebellar granule cell culture. Primary cultures of rat cerebellar granule neurons (CGCs) were prepared from 6-8 day old Long Evans rat pups as outlined by Gallo *et al*.[9] with modifications[10]. Cultures were grown in DMEM with 10% FBS and 30 mM KCl in 12-well plates (Corning Costar), with a plating density of 1.0×10^6 cells/ml. Cytosine arabinoside was added 48 hours after plating to prevent the proliferation of non-neuronal cells. Cultures were assayed at 7 days *in vitro* when they were fully developed.

Isolation of microsomes and mitochondria: Cerebella were excised rapidly from adult male Long Evans rats, and the fractionation was done according to Gray and Whittaker[11] and Dodd et al[12].

Intracellular signaling pathways. The effects of PBDEs and PCBs (0 to 30 μg/ml or 0 to 50 μM) were studied on the intracellular signaling pathways using cerebellar granule neurons or brain microsomes/mitochondria. The [^{3}H]AA release by CGCs into the media was determined according to the procedure modified from Lazarewicz *et al.*[13] and Tithof *et al*.[14]. The [^{3}H]-phorbol ester binding, which indicates PKC translocation from cytosol to the membrane, was determined according to the method of Vaccarino et al.[15]. The uptake of ^{45}Ca by microsomes and mitochondria was measured as outlined by Moore et al.[16].

Statistics. The data (n = 3-6 experiments, assayed in triplicates) were analyzed by a two-way analysis of variance (ANOVA) with chemical as one factor and concentration as the other using SigmaStat software, version 2.03 (SPSS Inc., Chicago, IL) . In the case of significant interaction, step-down ANOVAs were used to test for main effects of PBDEs or PCBs. Pair wise comparisons between groups were made using Fisher's LSD test. The accepted level of significance was $p < 0.05$.

Results and Discussion

PBDE effects on intracellular signaling pathways:

The mostly penta-BDE mixture, DE-71, stimulated [^{3}H]AA release in a concentration-dependent manner. A significant effect was seen at a concentration as low as 10 μg/ml (Table 1). The release of [^{3}H]AA by DE-71 is as early as 5 min of exposure and increased with time. Further results suggested that DE-71-induced [^{3}H]AA release is mediated by the activation of both Ca^{2+}-dependent and -independent cytosolic phospholipase A_2.

DE-71 also increased [^{3}H]PDBu binding in a concentration-dependent manner and a significant effect was seen at 3-10 μg/ml (Table 1). PBDE 47 increased [^{3}H]PDBu binding in a concentration-dependent manner with a significant effect at 10 μM. The effect seen with PBDE 47 was much greater than that of DE-71.

Table 1. The potency of PCBs and PBDEs on intracellular signaling processes in neuronal cultures and cerebellar fractions.

Intracellular signaling process	Lowest concentration with a significant effect			
	PBDE mixture (DE-71)	PCB mixture (Aroclor 1254)	PBDE congener (PBDE 47)	PCB congener (PCB 47)
Arachidonic acid release:	10 μg/ml	10 μg/ml	NT	NT
Phorbol ester binding:	3-10 μg/ml	3 μg/ml	10 μM	10 μM
Calcium buffering:				
Microsomes	10 μg/ml	3 μg/ml	NT	NT
Mitochondria	3 μg/ml	3 μg/ml	NT	NT

NT = not tested;

DE-71 also inhibited intracellular calcium buffering by both microsomes and mitochondria in a concentration-dependent manner. DE-71 inhibited mitochondrial ^{45}Ca-uptake to a greater extent when compared to microsomal ^{45}Ca-uptake (Tables 1 and 2).

Table 2. The efficacy of PCBs and PBDEs on intracellular signaling processes in neuronal cultures and cerebellar fractions.

Intracellular signaling process	% change in the effect at 30 μg/ml		% change in the effect at 50 μM	
	PBDE mixture (DE-71)	PCB mixture (Aroclor 1254)	PBDE congener (PBDE 47)	PCB congener (PCB 47)
Arachidonic acid release:	354 ± 75%↑	763 ± 73%↑	NT	NT
Phorbol ester binding:	29 ± 3%↑	142 ± 21%↑	75 ± 7%↑	91 ± 15%↑
Calcium buffering:				
Microsomes	49 ± 7% ↓	95 ± 1% ↓	NT	NT
Mitochondria	73 ± 6% ↓	96 ± 3% ↓	NT	NT

Values are mean ± SEM; NT= not tested;

Comparative effects of PCBs and PBDEs on intracellular signaling processes:
As observed before, Aroclor 1254, and PCB 47 perturbed all the selected signal transduction processes in a concentration-dependent manner. The potency of Aroclor 1254 seems to be almost similar to that of the PBDE mixture, DE-71 (Table 1). However, Aroclor 1254 was more efficacious than DE-71 on a weight basis (μg/ml) (Table 2). When the data were transformed on a molar basis, Aroclor 1254 and DE-71 were equally effective. PCB 47 and PBDE 47 increased [^{3}H]-PDBu binding to a similar extent on a molar basis (Tables 1 and 2).

Previously, we demonstrated that PCBs, which are known to cause developmental neurotoxicity, perturbed intracellular signaling processes[6,7,10] critical for nervous system development and associated with learning and memory processes. In the present study, PBDEs were shown to alter these signal transduction pathways with equal potency and efficacy on a molar basis. PBDEs are as ubiquitous as PCBs in human blood and breast milk samples[17], and the levels of PBDEs are rapidly rising in North Americans[18]. Considering the structural similarity of PBDEs with PCBs (Figure 1) and the known health effects of PCBs, these two groups of chemicals could conceivably work through the same mechanism(s), to cause developmental neurotoxicity. Due to the continued use of PBDEs in consumer products and their bioaccumulative nature, attention must be paid for the potential health risks associated with exposure to PBDEs.

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