COMPARATIVE EFFECTS OF PBDEs AND PCBs ON INTRACELLULAR SIGNALING IN RAT CEREBELLAR GRANULE NEURONS

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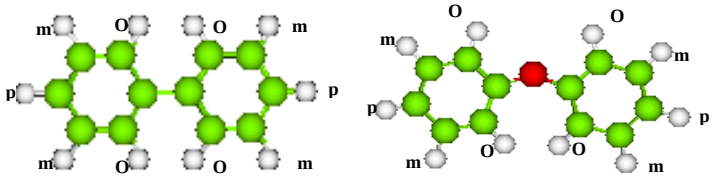
Introduction

Polybrominated diphenyl ethers (PBDEs) are synthetic chemicals that do not occur in nature and are structurally similar to polychlorinated biphenyls (PCBs; Figure 1) and several chlorinated pesticides. They are comprised of two phenyl rings linked by oxygen and are resistant to physical, chemical and biological degradation[1]. PBDE residues have been detected in indoor air, house dust, and foods[2,3]. PBDE exposure to humans may be possible via multiple sources (air, water, food and dust). PBDEs are found in higher levels in house dust in the United States than in Europe[4]. The predominant congeners detected are PBDE-47, PBDE-99 and PBDE-209. Levels of PBDEs in human tissues, specifically blood, milk, and fat, have increased exponentially since the 1970s in several countries, including the U.S Canada, and Sweden[5]. The doubling time for PBDE levels in the human body was estimated to be 3-5 years[6]. Currently in Sweden, PBDE levels in breast milk are decreasing, presumably as a result of a decrease in the use of PBDE-containing products. The EU has decreased PBDE use by two-thirds in recent years. Levels of PBDEs among individuals in North America, as measured in blood, breast milk, or adipose tissue, are 10-70 times higher than in Europe or Japan[5]. High levels of PBDEs in North America are attributed to the greater use of penta BDE mixture (>90%) when compared to the rest of the world. Like other lipophilic compounds, PBDEs readily cross the placenta into the fetus. This provides the opportunity for PBDEs to interfere with developmental processes, possibly producing developmental effects.

In spite of their widespread occurrence in the environment, only limited information is available on the toxicology of PBDEs in terms of their mode of action. Previous studies showed that PBDE exposure caused aberrations in spontaneous behavior and reduced learning and memory in mice; these effects are similar to those seen after exposure to DDT or PCBs[7-9]. However, the mode of action for this group of chemicals remains unclear. The underlying molecular mechanisms of the adverse health effects of PCBs have been associated with perturbations in intracellular signaling mechanisms including Ca^{2+} homeostasis, [^{3}H]arachidonic acid ([^{3}H]AA) release, mitogen-activated protein kinase (MAPK) activation, and translocation of protein kinase C[10] (PKC). These intracellular signaling events are critical for learning and memory, normal function and development of the nervous system[11-12]. We have extended studies to understand whether PBDEs affect intracellular signaling in a similar way to those of PCBs. The objective of this study is to compare the efficacy and potency between PCB and PBDE mixtures, mixtures versus congeners, and among congeners.



Biphenyl Ring **Diphenyl Ether Ring**

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 (West Lafayette, IN). 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). The final concentration in the assay buffer (0.2 to 0.4% DMSO 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.*[13] with modifications[14]. 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[15] and Dodd et al[16].

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.*[17] and Tithof *et al.*[18]. 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.[19]. The uptake of ^{45}Ca by microsomes and mitochondria was measured as outlined by Moore et al.[20]. The quantification of total and phospho-MAPK (ERK1/2) was done by western blots using specific antibodies[21].

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 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.

DE-71 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).

DE-71 increased phospho-ERK1/2 in a concentration-dependent manner with a significant effect starting at 3 μg/ml. PBDE 47 also increased phospho-ERK1/2 in a concentration-dependent manner with a significant effect at 1 μM. The effect seen with PBDE 47 was much greater than that of DE-71 (Tables 1 and 2).

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
p-ERK1/2 activation	3 μg/ml	1 μg/ml	1 μM	3 μM

NT = not tested;

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
p-ERK1/2 activation	480 ± 100 ↑	415 ± 105 ↑	383 ± 19 ↑	295 ± 58 ↑

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) on [^{3}H]AA release, PKC translocation and calcium buffering while equally efficacious in phosphor-ERK1/2 activation. (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[10] critical for nervous system development and associated with learning and memory processes. In the present study, PBDEs were shown to alter similar signal transduction pathways with almost equal potency and efficacy on a molar basis. PBDEs are as ubiquitous as PCBs in human blood and breast milk samples[22], and the levels of PBDEs are rising in North Americans[23]. 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 similar mechanism(s), to cause developmental neurotoxicity. Due to continued presence of these two groups of chemicals in human food and environment, the potential interaction between these chemicals and the adverse effects associated with their exposure may be important in their risk assessment.

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This abstract does not necessarily reflect USEPA policy.

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