DIFFERENTIAL EFFECTS OF POLYBROMINATED DIPHENYL ETHERS AND POLYCHLORINATED BIPHENYLS ON [^{3}H]ARACHIDONIC ACID RELEASE IN RAT NEURONAL CELLS

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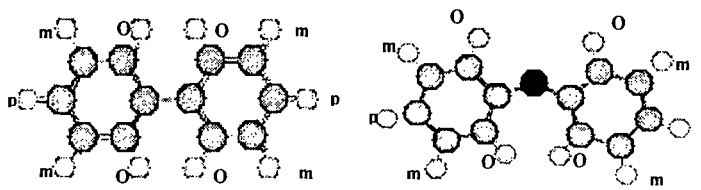
Introduction

Polybrominated diphenyl ethers (PBDEs) are produced and used in large quantities as flame retardants in electrical equipment, plastics, and building materials. The global production is in the range of 80 million pounds annually[1] and become ubiquitous contaminants because of high production, lipophilic characteristics and persistence. PBDEs have similar chemical structure and physicochemical properties (e.g.,high lipophilicity and low reactivity) to that of other persistent pollutants such as PCBs and dioxins (Figure 1). PBDEs have been detected in human blood, adipose tissue and breast milk, and long-term exposure during development to these contaminants 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 milk 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 widespread occurrence in the environment, extremely limited information is available on the toxicology of these chemicals. Recent studies demonstrated that PBDE exposure can cause aberrations in spontaneous behavior and reduced learning and memory in mice[3-5]; the effects are similar to the ones seen after exposure to DDT or PCBs[6]. However, the mode of action remains unclear.

Previously, we demonstrated that PCBs, which are structurally similar and known to cause neurotoxic effects, stimulated the release of [^{3}H]arachidonic acid ([^{3}H]AA) by a $cPLA_2$/$iPLA_2$ dependent mechanism[7]. PLA_2 activity has been associated with learning and memory, and AA has been identified as a second messenger involved in synaptic plasticity[8]. The objectives of the present study are: (a) test whether PBDE mixtures (DE-71 and DE-79) have a mode of action similar to that of PCBs and other organohalogens; (b) compare the effects of two PBDE mixtures,

DE-71 and DE-79, on [^{3}H]arachidonic acid release; (c) delineate the possible mechanism(s) by which PBDEs stimulated the release of [^{3}H]arachidonic acid release.



Biphenyl Ring Diphenyl Ether Ring

FIG. 1: Structural features of polychlorinated biphenyls and polybrominated diphenyl ethers

Materials and Methods

Chemicals. Radiolabeled [5, 6, 8, 9, 11, 12, 14, 15-^{3}H(N)]arachidonic acid (210 Ci/mmol; >97% pure) was purchased from Dupont NEN Corporation (Boston, MA). PBDE mixtures were a gift from Dr. Kevin Crofton, PCB mixtures were purchased from AccuStandard (New Haven, CT), and dissolved in dimethyl sulfoxide (DMSO). The final DMSO concentration in the assay buffer did not exceed 0.4% (v/v). DMSO at this concentration did not significantly affect [^{3}H]AA release.

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, Kodavanti *et al.*[10]. Cultures were grown in DMEM with 10% FBS and 30 mM KCl in 12-well plates (Corning Costar), with a plating density 1.5 x 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 are fully developed (Figure 2).

[^{3}H]Arachidonic Acid (AA) Release. The [^{3}H]AA release by CGCs into the media was determined according to the procedure modified from Lazarewicz *et al.*[11] and Tithof *et al.*[12]. Cells were labeled for 16-20 hours with 1 µCi [^{3}H]AA per well. The cells were then washed with modified Locke's buffer once and twice with modified Locke's + 0.2% BSA. Preincubation was for 10 min in modified Locke's + 0.2% BSA with or without the addition of pharmacological agents. The cells were exposed for 20 min to PBDE mixtures in the presence or absence of pharmacological agents in modified Locke's + 0.2% BSA (1 ml) with or without extracellular calcium (without Ca^{2+}, 0.3mM EGTA). The media was immediately removed after exposure for counting in scintillation counter, and 1 ml of 0.5 N NaOH was added to lyse the cells to measure total incorporation of [^{3}H]AA.

Statistics. Data (mean ± SEM of 3-5 preparations, assayed in triplicate) were expressed as a % of total cellular radioactivity incorporation per well. The data were analyzed by a two-way analysis of variance (ANOVA). In the case of significant interaction, step-down ANOVAs were used to test for main effects of PBDEs or pharmacological agents. Pair wise comparisons between groups were made using Dunnett's t-test[13]. The accepted level of significance was $p < 0.05$.

Results and Discussion

PBDE effects on [^{3}H]AA release:

The effects of two PBDE mixtures with different congener compositions were studied on [^{3}H]AA release by cerebellar granule neurons. 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. On the other hand, a mostly octa-BDE mixture, DE-79, did not stimulate [^{3}H]AA release even at 50 μg/ml (Figure 3). The release of [^{3}H]AA by DE-71 is as early as 5 min of exposure and increased with time (Figure 4).

Removal of extracellular calcium and chelation by 0.3 mMEGTA significantly decreased the DE-71 stimulated [^{3}H]AA release; however only a 30 % inhibition of the release was demonstrated for the calcium replete conditions at 30 μg/ml DE-71 (Figure 5). These results suggest that extracellular calcium plays a role in the DE-71 effects on[^{3}H]AA release. Methyl arachidonyl fluorophosphonate (MAFP) at 5 μM significantly attenuated DE-71 stimulated [^{3}H]AA release (Figure 6). MAFP inhibits both Ca^{2+}-dependent and -independent cytosolic phospholipase A_2 ($cPLA_2$)[14]. The attenuation by MAFP suggests that DE-71stimulated release of [^{3}H]AA may occur through $cPLA_2$. Previously, we demonstrated that other structurally related chemicals such as PCBs increased intracellular free calcium[15] which is dependent upon the presence of extracellular calcium[16]. We have also demonstrated that removal of extracellular Ca^{2+} caused a slight, but significant decrease in PCB-stimulated [^{3}H]AA release. Both calcium-independent and -dependent cPLA are present in the rat cerebellum[17] and one or both are probably involved in the stimulation of [^{3}H]AA release in rat cerebellar granule cells by PCBs, PBDEs, and other organohalogens. These results indicate that PBDEs stimulated [^{3}H]AA release by activating the PLA_2 pathway as do other organohalogen mixtures.

Comparative effects of PCBs and PBDEs on [^{3}H]AA release:

The selected PCB mixtures, Aroclor 1016 and Aroclor 1254, stimulated [^{3}H]AA release in a concentration-dependent manner (Figure 7). The effect was much greater when compared to PBDEs. PCB mixtures also initiated the response much earlier than PBDEs. Although there are some quantitative differences, the effects of PBDEs are qualitatively similar to those of PCBs. Hence, attention must be paid for the potential risk associated with exposure to PBDEs.

Acknowledgments

Authors thank Dr. Stephanie Padilla for her comments on the earlier version of this abstract. This abstract does not necessarily reflect USEPA policy.

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FIG. 2. Cerebellar granule cells at 7 days *in vitro* (x 360). Cells are fully differentiated by 6 days and all experiments were conducted on 7 days *in vitro*.

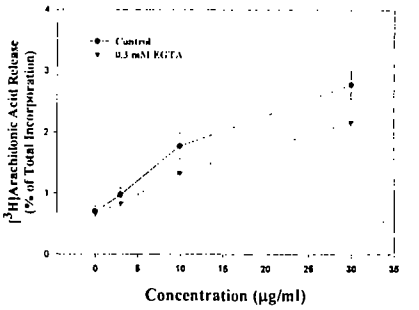

FIG. 5: DE-71 stimulated release of [^{3}H]AA in the presence (control) and absence (presence of 0.3 mM EGTA) of external Ca^{2+}.

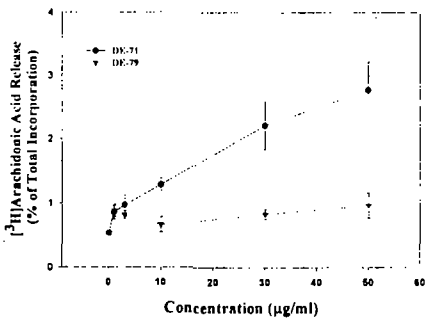

FIG. 3: Effects of PBDE mixtures (DE-71 and DE-79 on [^{3}H]AA release.

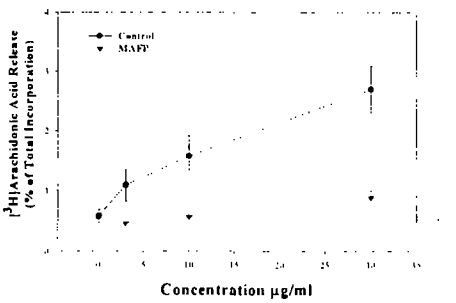

FIG. 6: Methyl arachidonyl fluorophosphonate (MAFP) inhibition of DE-71 (30 μg/ml) stimulated [^{3}H] AA release.

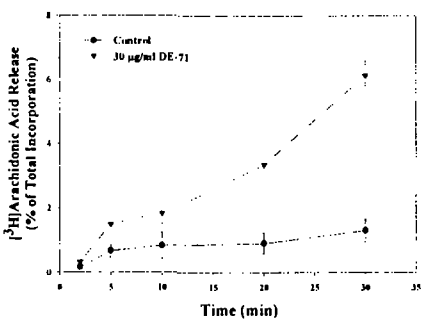

FIG. 4: Time-course of [^{3}H]AA release following exposure to DE-71.

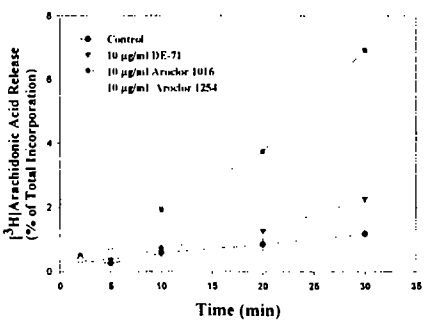

FIG. 7: Comparison of time-course of [^{3}H]AA release by PCB and PBDE mixtures.