The effect of brominated flameretardants on membranes from the central nervous system

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Introduction

Brominated flame retardants (BFRs, Fig 1) are used in a wide range of manufactured products, and their distribution in the environment is a cause of major concern. The properties of BFRs are similar to other persistent lipophilic organic compounds and have the potential to bio accumulate in the food web (1-4). The environmental levels of the BFRs are still generally lower than for PCB, however, the environmental levels are increasing, which is a matter of concern since the annual demand of brominated flame retardants is increasing. It is claimed that the BFRs share some of the same toxic effects as shown for the chlorinated organic compounds, such as the PCBs (5-6). The PCBs are previously established as neurotoxicants (7-10). To indicate a toxic potential of the BFRs we have therefore screened the effect of different technical mixtures of BFRs on different nerve preparates.

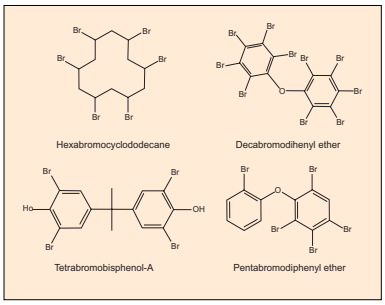

Figure 1: *Chemical structure of four of the mostly used BFRs.*

Materials and Methods

The BFRs pentabromodiphenyl ether (PBDE), octabromodiphenyl ether (OBDE), decabromobiphenyl ether (DBDE), hexabromobiphenyl (HBB), octabromobiphenyl (OBB), hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBP-A), 2, 4, 6-tribromophenyl allyl ether (TBPAE), and 2,2-bis (4-(2,3-dibromopropoxy-3,5 dibromophenyl) - propane (BDBBP) were purchased from Promochem (Sweden).

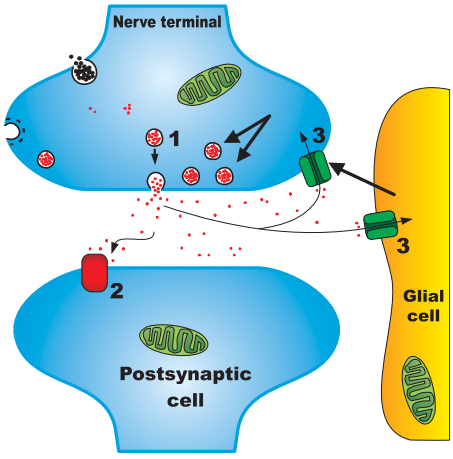

Figure 2: *Different compartments of a nerve terminal with an adjacent glial cell. Upon nervous stimulation neurotransmitters are released from synaptic vesicles (1). Neurotransmitters then interact with receptors (2). The response of transmitters is stopped by reuptake into the nerve terminal (3) or adjacent glial cells, followed by reuptake into the synaptic vesicles.*

Synaptosomes, which are pinched off nerve terminals (Fig 2), were isolated as described previously (7).

Synaptic vesicles, which are the storing compartments of neurotransmitter in the nerve terminal (Fig 2), were isolated on a sucrose density gradient (9).

The effect of different BFRs on the uptake of neurotransmitters into synaptosomes and synaptic vesicles were performed as described previously (7,9).

The effect of BFRs on cerebellar granule cells, which is a primary cell culture from rat cerebellum (Fig 3), was performed as described (10). Neuron survival was determined by using the trypan blue exclusion assay.

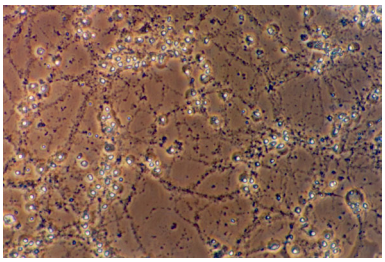

Figure 3: *Cerebellar granule cells are primary cell culture isolated from rat cerebellum.*

Conclusion

This investigation shows that BFRs have toxic effects on membrane preparates in a concentration range even lower than observed for the PCBs (μM).

Synaptosomal membranes: Two of the mostly used BFRs, HBCD and TBBP-A, inhibited neurotransmitter uptake into nerve terminals in a concentration range similar to PCB and the party drug ecstasy, indicating a toxic potential (Table 1, Fig 4). HBCD was a more selective uptake inhibitor than TBBP-A indicating that TBBP-A has a more pronounced effect on the properties of the cell membrane fluidity influencing the generally uptake mechanisms.

Synaptic vesicles: HBCD, TBBP-A and PBDE, claimed to be the most toxic BFR, inhibited uptake of dopamine into synaptic vesicles (Fig 5).

Cell culture: HBCD, TBBP-A and PBDE also induced a concentration dependent death of the cerebellar granule cells (Fig 6).

These findings suggest that several BFRs have a toxic potential and confirm the importance of monitoring the levels of BFRs in the environment, especially HBCD, which is used as an additive BFR.

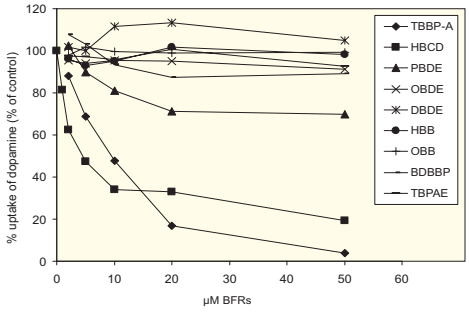

Figure 4: *BFRs inhibit the uptake of dopamine into pinched off nerve terminals (synaptosomes).*

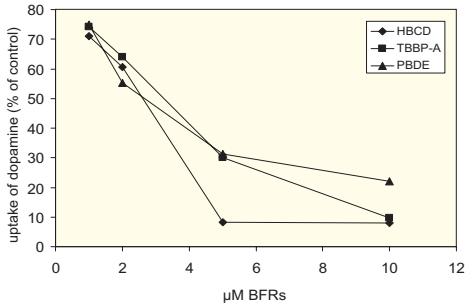

Figure 5: *BFRs inhibit the uptake of dopamine into synaptic vesicles (the storing organelle for neurotransmitters in the neuron).*

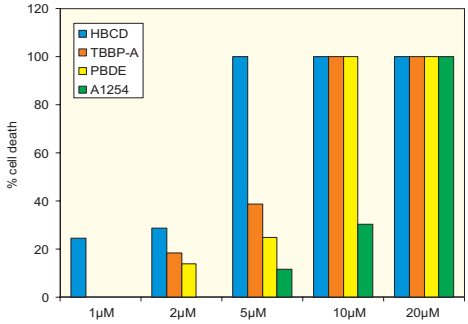

Figure 6. *Death of cultured rat cerebellar granule cells after exposure to increasing doses of the BFRs HBCD, TBBP-A, PBDE and the PCB mixture A1254 for 24h. Values are presented as % cell death.*

Table 1: *Estimated IC_{50} values of the uptake of dopamine, glutamate and GABA into synaptosomes and synaptic vesicles. For comparison the effect of the PCB mixtures Aroclor 1242 and 1254, and the dopamine uptake inhibitor ecstasy are included in the table. The values are presented in μM.*

	Synaptosomes			Vesicles
Substances	dopamine	glutamate	GABA	Dopamine
TBBPA	10	5	15	3
HBCD	5	50	-	3
PBDE	>50	>50	>50	4
A1242(7)	7	5	6	
A1254(7)	4	4	4	
Ecstasy*	2	-	-	5

* The values were kindly provided by Kristin Huse Haug and Dr Oddvar Myhre, NDRE, (unpublished results).

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