Effects of a commercial pentabrominated diphenyl ether mixture on cholinergic neurochemical biomarkers in mink

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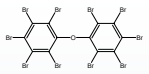
Abstract

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants (BFRs) that have been identified as global environmental and human contaminants and have generated growing health concerns. They have been shown to elicit neurodevelopmental toxicity through the cholinergic neurotransmitter system in rodents (Viberg *et al*. 2002, 2003, 2004; Dufault *et al*. 2005), however the evidence for the behavioral effects is much better corroborated than that for the neurochemical effects. The objective of this study was to assess the effects of dietary exposure to the commercial pentabrominated diphenyl ether mixture DE-71 on cholinergic neurochemical biomarkers in farmed mink (*Mustela vison*). A 1-generation DE-71 mink feeding trial was conducted, from which adult females, kits weaned at six weeks of age and juveniles at 32 weeks of age were sampled for blood and necropsied. Neurochemical biomarkers of the cholinergic neurotransmitter system, including muscarinic acetylcholine receptor (mAChR), nicotinic acetylcholine receptor (nAChR), acetylcholine (ACh) and cholinesterase (ChE) were assayed in cerebral cortex, and ChE measured in plasma. Results indicated effects of DE-71 on reproduction (no kits whelped at the highest dose) and possibly on liver function (a three-fold elevation in plasma ChE activity of adult females at the highest dose), but no significant effects of DE-71 on mAChR, nAChR, ACh or ChE in cerebral cortex. The direct effects of DE-71 on the function of mAChR, nAChR and ChE *in vitro* were also assessed in whole brain, but results showed no effects of DE-71 on these biomarkers. This study demonstrated that DE-71 did not have any effects on cholinergic neurochemical biomarkers in mink brain, neither *in vivo* nor *in vitro*.

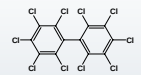
Introduction

PBDEs

- Non-coplanar PCBs are toxic to the developing brain, in particular through the cholinergic neurotransmitter system (Eriksson *et al*. 2001)
- PBDEs share structural similarity with non-coplanar PCBs
- Evidence for the neurodevelopmental toxicity of PBDEs through the cholinergic neurotransmitter system is limited, in particular for the neurochemical effects



Decabrominated diphenyl ether (PBDE)

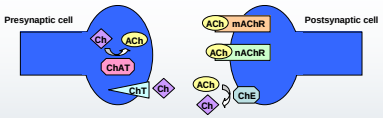


Decachlorinated biphenyl (PCB)

Mink (*Mustela vison*)

- Excellent sentinel species to assess environmental health (Basu 2005)
- Distributed across North America and can be studied in captivity
- Piscivores, bioaccumulate contaminants, sensitive to PCBs (Bursian *et al*. 2005) and PBDEs (Martin *et al*. 2004)

Cholinergic neurotransmitter system



Neurotransmitter
Acetylcholine (ACh)

ACh Synthesis
Choline acetyltransferase (ChAT)

Ch Uptake
Choline transporter (ChT)

ACh Receptors
Muscarinic receptor (mAChR)
Nicotinic receptor (nAChR)

ACh Degradation
Cholinesterase (ChE)

Objectives

1. To assess the effects of dietary exposure to DE-71, a commercial pentabrominated diphenyl ether mixture (Great Lakes Chemical Corporation, USA), on cholinergic neurochemical biomarkers in ranch mink (*in vivo* study)
2. To assess the direct effects of DE-71 on the function of cholinergic neurochemical biomarkers in ranch mink (*in vitro* study)

Methodology

1-GENERATION DE-71 MINK FEEDING TRIAL

4 dose groups: 0, 0.1, 0.5, 2.5 μg/g DE-71 in feed

3 age groups:

- Adult females: 10 per dose group, fed treatment diets from 4 weeks before breeding to weaning of kits at 6 weeks of age
- 6-week-old kits: 6 per dose group, weaned
- 32-week-old juveniles: 10 per dose group, fed treatment diets from weaning at 6 weeks of age to 32 weeks of age

Brain tissue and blood samples collected, cerebral cortex and plasma isolated

CHOLINERGIC NEUROCHEMICAL BIOMARKER ASSAYS

mAChR and nAChR assay

- mAChR and nAChR specific binding (total – non-specific) were measured using a 96-well 0.22 μm GF/B glass filter system (Millipore, Boston, MA, USA)
- Total binding: Samples were incubated with 1 nM [^{3}H]-QNB (a mAChR-specific radioligand) or [^{3}H]-CYT (a nAChR-specific radioligand)
- Non-specific binding: Samples were pre-incubated with 100 μM atropine (a mAChR-specific antagonist) or nicotine (a nAChR-specific antagonist) then incubated with radioligand
- Incubation was terminated by filtration and radioactivity retained on filter was measured

ACh and ChE assay

- ACh concentration and ChE activity were measured using a fluorescent (Amplex Red) microplate method (Zhou *et al*. 1997, Zhou *et al*. 2000)

***In vivo* study**

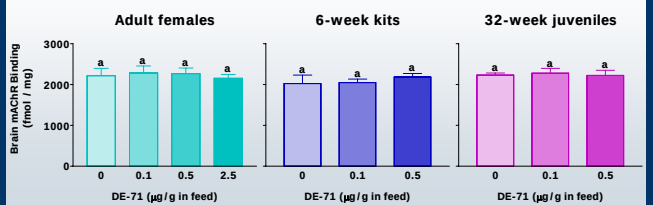
- mAChR, nAChR, ACh and ChE were measured in cerebral cortex, ChE was measured in plasma

***In vitro* study**

- mAChR, nAChR and ChE were measured in whole brain incubated with DE-71 (9 concentrations, 0-13.3 μg/L)

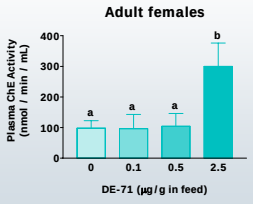
Results

Effects of DE-71 on mAChR binding in mink cerebral cortex *in vivo*

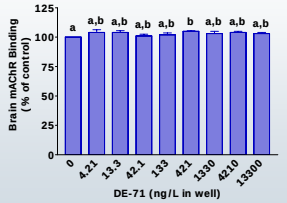


Results (continued)

Effects of DE-71 on ChE activity in mink plasma *in vivo*



Effects of DE-71 on mAChR binding in mink whole brain *in vitro*



- Effects of DE-71 on remaining biomarkers in mink brain and plasma (*in vivo*) and mink brain (*in vitro*) not significant

Conclusions

EFFECTS OF DE-71

Reproduction

- Adult females conceived but no offspring whelped in 2.5 μg/g dose group

Cholinergic neurochemical biomarkers

- No significant differences in mAChR binding, nAChR binding, ACh concentration or ChE activity with DE-71 dose in cerebral cortex (*in vivo*)
- No significant differences in the function of mAChR, nAChR or ChE with DE-71 concentration in whole brain (*in vitro*)

Liver function (possibly?)

- 3-fold increase in plasma ChE activity of adult females in 2.5 μg/g dose group
- Increased synthesis and/or secretion of ChE? Necrosis?

Significance

- Neurodevelopmental toxicity of PBDEs through cholinergic neurotransmitter system not observed
- No offspring in 2.5 μg/g dose group but doses (μg/g in feed) up to 1000-fold higher than environmentally relevant concentrations (ng/g in fish) (Law *et al*. 2003)
- Results from this study not consistent with those of Viberg *et al*. (2003, 2004), therefore more studies needed to assess the neurochemical effects of PBDEs on the cholinergic neurotransmitter system

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