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 6 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 weaned 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

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

Cholinergic neurotransmitter system

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Acetylcholine (ACh)</th>
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<tr>
<td>ACh Receptors</td>
<td>Muscarinic receptor (mAChR)</td>
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<td></td>
<td>Nicotinic receptor (nAChR)</td>
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<td>ACh Synthesis</td>
<td>Choline acetyltransferase (ChAT)</td>
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<td>Ch Uptake</td>
<td>Choline transporter (ChT)</td>
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<tr>
<td>ACh Degradation</td>
<td>Cholinesterase (ChE)</td>
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Methodology
1-GENERATION DE-71 MINK FEEDING TRIAL
4 dose groups: 0, 0.1, 0.5, 2.5 μg 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 from weaning at 6 weeks of age to 32 weeks of age
- 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

Conclusions
EFFECTS OF DE-71
Reproduction
- Adult females conceived but no offspring weaned 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 (μg/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

Results
EFFECTS OF DE-71 ON mAChR BINDING IN MINK BRAIN IN VITRO
- Effects of DE-71 on mAChR binding in mink cerebral cortex in vivo
- No offspring in 2.5 μg/g dose group but doses (μg/g in feed) up to 1000-fold higher than environmentally relevant concentrations (μg/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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