Uptake of PBDEs in pike (*Esox lucius*) from food

Sven Burreau and Dag Broman

Aquatic Chemical Ecotoxicology
Department of Zoology
Stockholm University
S-106 91 Stockholm
Sweden

Introduction

During the last years polybrominated diphenyl ethers (PBDEs) have been of interest from an ecotoxicological point of view. PBDEs are used for fire protection of textiles and plastics and have been found in biotic and abiotic samples from the environment in different parts of the world [1-4]. The PBDE pollution can take place when PBDEs, which are additive flame retardants, leak from the materials in which they are used to the environment [5]. Nylund et al [6] reported that the levels of PBDEs in sediments from Baltic Sea have increased rapidly since 1980. The chemical and structural similarity of the PBDEs to well known contaminants, e.g. polychlorinated biphenyls (PCBs), make it interesting to establish their ecotoxicological fate. For this reason we have conducted food exposure experiments of three PBDEs using pike as test organism. By doing this we wanted to show the extent of PBDE assimilation in pike and the tissue distribution of PBDE after food exposure.

Materials and methods

This study includes two parts: uptake efficiency of three PBDEs (Experiment 1) and tissue distribution of PBDE #47 (Experiment 2). All test substances were provided by Department of Environmental Chemistry, Stockholm University and had been synthesised according to the methods described in [7].

Experiment 1 - Uptake efficiency

This part of the study has been published [8] and the methods are only briefly described here. Pike were fed with rainbow trout (*Onchorhynchus mykiss*) in which the test substances (PBDE #47, 99 and 153), dissolved in lipid extract from rainbow trout, had been injected in the dorsal muscle. The substances were after extraction and clean-up analysed using GC/MS. Uptake efficiency (E) values of the substances were calculated as the amount in the pike divided by the exposure amount.

Experiment 2 - Internal distribution
The exposure method was essentially the same as in Experiment 1 but the test substance was ^{14}C-labelled PBDE #47. The radioactivity given to the pike was between 0.21 and 0.24 μCi/g (fresh weight). The pike were sacrificed after 9, 18 and 36 days after which they were analysed using whole-body autoradiography as described in [9]. One pike that died after 65 days was also analysed. Some tape sections were extracted as described in [10] to investigate if there occurred any covalent binding of radioactivity. Two additional pike, one exposed and one non-exposed pike, were controlled for radioactivity using scintillation counting. The exposed pike that was analysed with scintillation counting had twice been fed after the exposure feeding.

Results and Discussion
Experiment 1 - Uptake efficiency
The uptake efficiency (E) values are shown in Fig 1. The PBDEs efficiently absorbed from the food, especially PBDE #47. This compound was assimilated much more efficiently than PCBs analysed in the same study (data not shown here).

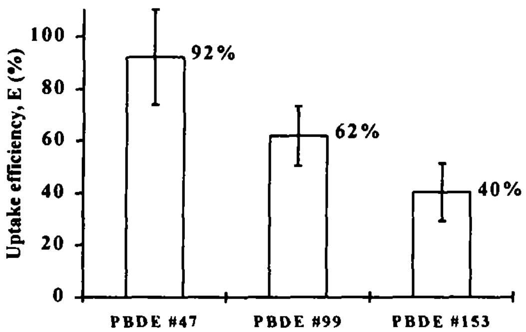

Fig 1. *Uptake efficiency (E) of the three PBDEs*

The uptake efficiency is negatively correlated with the degree of bromination and hence with the hydrophobicity and the molecular weight of the compounds. The high uptake efficiencies, especially for PBDE #47 and #99, are in conflict with a study by Gobas et al. [11] who reported a maximum uptake efficiency for hydrophobic organic compounds (HOCs) at <50% and a decline in E with hydrophobicity for substances with log Kow >6.5. A possible explanation for the discrepancies between different food exposure studies of HOCs could be the different exposure procedures. Often, the uptake of HOCs in fish after dietary exposure is concluded to occur through diffusion from the gastro intestinal (GI) tract to the fish. However other mechanisms, such as endocytosis, could be responsible for the HOC uptake. Examples of active and protein mediated lipid uptake have been shown in several vertebrates [12-14]. Such non-diffusive uptake may vary with the quality of the food. If this is the case it is really important to use exposure methods that mimic the "natural" situation in order to make the right conclusions from dietary exposure studies of HOCs.

It has been suggested that compounds with an effective cross section (ECS) > 9.5 Å do not pass biological membranes in fish gills [15] and possibly also in the GI tract of fishes [16]. This is not the case in the present study. PBDE # 99 and 153 are both absorbed in spite of their ECS that is 9.6 Å. Again, this could be due to the exposure method. It might be that when dissolving the test substances in lipid extract, instead of adsorbing them on artificial food pellets, it is made possible for the substances to be co-assimilated the with lipids.

Experiment 2 - Internal distribution
The results from the scintillation counting of the exposed pike confirmed the high E value for PBDE #47 from Experiment 1. The scintillation counting showed an E value for this substance at 96 %. The radioactivity of the non-exposed pike did not differ from the background (i.e. 0.002 of the radioactivity of the exposed pike). The autoradiographic results show that radioactivity derived from PBDE #47 was distributed to all parts of the body, including the brain and the spinal chord. One tape section autoradiogram from the pike that was sacrificed after 9 days is shown in Fig 2.

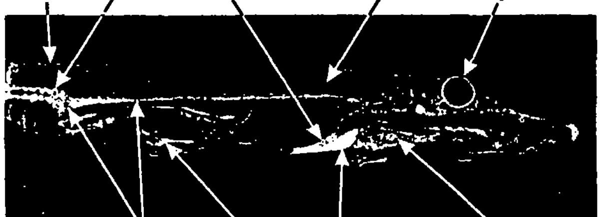

Fig 2. *Tape section radiogram of a pike 9 days after dietary exposure with ^{14}C-labelled PBDE #47. The brain and spinal chord are not present in this section.*

The distribution of radioactivity can to a part be explained with the difference in lipid content of the different organs. The presence of radioactivity in the brain and the spinal chord indicate that PBDE #47 (or some relatively hydrophobic metabolite of this substance) is not prevented by the blood-brain barrier. The high radioactivity of the vertebrae surrounding tissue (VST) was unexpected. In other teleost species such as flounder (*Platichthys flesus*) [17] and carp (*Cyprinus carpio*) [18] there is a minor lipid deposit surrounding the backbone that has been shown to accumulate different HOCs. This might be the case in the pike too. The high radioactivity in the eye capsule and the kidney might be explained with the melanin content of these organs. Melanin has been found to bind several organic compounds [17, 19, 20]. After the longer periods, the levels were clearly diminished in the muscle tissue but no decrease in the most lipid rich tissues were observed after 36 and 65 days.

There were traces of radioactivity in the extracted tape sections, especially in the VST, but it is concluded that this is explained by a non-exhaustive extraction rather than covalent binding to macromolecules since the radioactivity traces are much weaker than in studies in which covalent binding has been shown [21, 22].

In conclusion PBDE #47, 99 and 153 are efficiently absorbed from the food in pike. The uptake efficiency (E) is negatively correlated with the degree of bromination. The highest E value, > 90%, was observed for PBDE #47. The autoradiographic pattern indicate that PBDE #47 or possibly some relatively hydrophobic metabolite is retained in the most lipid rich tissues of the pike a considerable time.

References

1. Andersson Ö and Blomkvist G; Chemosphere. **1981**, 10, 1051-1060.
2. Jansson B Asplund L and Olsson M; Chemosphere. **1987**, 16, 2343-2349.
3. Watanabe I Kashimoto T and Tatsukawa R; Chemosphere. **1987**, 16, 2389-2396.
4. de Boer J; Chemosphere. **1989**, 18, 2131-2140.
5. Bergman Å; Proceedings, *Workshop on brominated aromatic flame retardants*. **1989**. Skokloster, Sweden: National Chemicals Inspectorate, Sweden.
6. Nylund K Asplund L Jansson B Jonsson P Litzen K and Sellström U; Chemosphere. **1992**, 12, 1721-1730.
7. Örn U Eriksson L Jakobsson E and Bergman Å; Acta Chem. Scand. **1996**, 50, 802-807.
8. Burreau S Axelman J Broman D and Jakobsson E; Environ. Toxicol. Chem. **1997**, 16, 2508-2513.
9. Ullberg S; Acta Radiol. **1954**, Suppl. 118, 1-110.
10. Brandt I and Brittebo E B, p. 295-314, in *Intermediary xenobiotic metabolism in animals. Methodology, mechanisms and significance*, Ed. D.H. Hutson, J. Caldwell, and G.D. Paulson, **1989**; Taylor & Francis: London New York Philadelphia.
11. Gobas F A P C Muir D C G and Mackay D; Chemosphere. **1988**, 17, 943-962.
12. Stremmel W; J. Clin. Invest. **1988**, 82, 2001-2010.
13. Thurnhofer H and Hauser H; Biochemistry. **1990**, 29, 2142-2148.
14. Thomson A B R Schoeller C Keelan M Smith L and Clandinin M T; Can. J. Physiol. **1993**, 71, 531-555.
15. Opperhuizen A d. Velde E Gobas F A P C Liem D A K and d. Steen J M; Chemosphere. **1985**, 14, 1871-1896.
16. Opperhuizen A and Sijm D T H M; Environmental Toxicology and Chemistry. **1990**, 9, 175-186.
17. Solbakken J E Ingebrigtsen K and Palmork K H; Mar. Biol. **1984**, 83, 239-246.
18. Yoshida T Takashima F and Watanabe T; Ambio. **1973**, 2, 111-113.
19. Horsberg T E Martinsen B and Varma K J; Aquaculture. **1994**, 122, 97-106.
20. Larsson B S and Roberto A; *Melanin affinity of carcinogenic substances as a risk factor of malignant melanoma*. Report., Dept. of Toxicology: Uppsala. **1992**
21. Brandt I Jönsson C-J and Lund B-O; Ambio. **1992**, 21, 602-604.
22. Jönsson C-J Lund B O Brunström B and Brandt I; Environ. Toxicol. Chem. **1994**, 13, 1303-1310.