METABOLISM AND DISTRIBUTION OF 2,2',4,4'-TETRABROMO[^{14}C]DIPHENYL ETHER IN PIKE (*Esox lucius*) AFTER DIETARY EXPOSURE

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Introduction

Polybrominated diphenyl ethers (PBDE), commonly used as flame retardants, are widely distributed in the environment. PBDE have been found to bioaccumulate and biomagnify in wildlife (for a review see [1]). Although higher brominated technical products (octa- and decabrominated) are the most frequently used, the most abundant congener in biota is 2,2',4,4'-tetrabromodiphenyl ether (BDE47) i.e. one of the major components in pentabrominated technical formulas. BDE47 appears to bioaccumulate to a large extent and also shows the highest uptake efficiency in fish [1, 2].

In analogy to the metabolism of PCB, hydroxylated PBDEs have been indicated in rats and mice exposed to PBDE congeners [3,4]. *Para*-hydroxylated PBDEs have structural similarities to the thyroid hormone thyroxine, which is carried by transthyretin, a plasma transport protein, to the target organs. Metabolites, produced in *in vitro* systems, of a number of BDE congeners including BDE47 were potent competitors to thyroxine for binding to human transthyretin [5]. Also, the levels of thyroxine decreased in rats and mice treated with BDE47 [6]. Furthermore, BDE47 has been shown to induce permanent abberations in the motor behaviour in mice exposed neonatally [7].

Little is known about the metabolism of PBDE in wildlife species. Both hydroxylated and methoxylated PBDEs have been reported in Baltic Salmon blood plasma [8]. The aim of this study was to learn more about the metabolism of BDE47 in fish exposed via food to mimic natural exposure.

Methods and Materials

^{14}C-BDE47 was synthesized as previously described [9]. Pike (n=23, mean weight 47,4 ± 7,8 g) were kept at 14 °C in separate aquaria. The method used for exposure has been described elsewhere [2]. In short either unlabelled BDE47 or ^{14}C-labelled BDE47 (specific activity 1 Ci/mole) was dissolved in trout lipids, injected into the dorsal muscle of live trout, which was instantly given to the pike. The dose was 1.1 µmole/fish. After 9 and 18 days, 9 and 11 pike respectively, were sacrificed. Liver, spleen, heart, brain, head and trunk kidney, gills, muscle, the tissue surrounding the spinal cord (VST), intestinal mucosa, blood and gall bladder were collected, and kept frozen until the time of analysis.

Muscle tissues were analysed individually, all other tissues/organs were pooled into four groups; labelled and unlabelled BDE47 at 9 and 18 days respectively. The samples were homogenised and solvent extracted with acetone: hexane according to [10]. Aliquots of muscle extracts were treated with concentrated sulphuric acid for further quantification of BDE47 with GC/MS. All extracts were fractionated by HRGPC into 3 major fractions; a lipid fraction, a PBDE fraction including the

parent compound and a non-conjugated metabolite fraction [8]. The metabolite fraction was further separated into a phenolic and a neutral fraction by partitioning with KOH [8]. The phenolic fraction was derivatised with diazomethane prior to GC/MS analysis. For identification purposes, several hydroxylated PBDEs were synthesised [11]. The samples were analysed by GC/MS (ECNI) monitoring the bromide ions. Radioactivity was measured by liquid scintillation counting in fresh tissues, extracts, and in residues produced during extraction and clean-up. All concentrations presented are normalised to the mean weight of the exposed fish in order to compensate for the differing body weights of the individuals.

Results and Discussion

In an autoradiography study on pike exposed to ^{14}C-BDE47, using similar experimental conditions the uptake was estimated to 96% [12]. In the present study the mean concentration of BDE47 in muscle extracts, determined by GC/MS was 230 ± 98µg/g lp w at 9 days (n=9) and 240 ± 81µg/g lp w at 18 days (n=8).

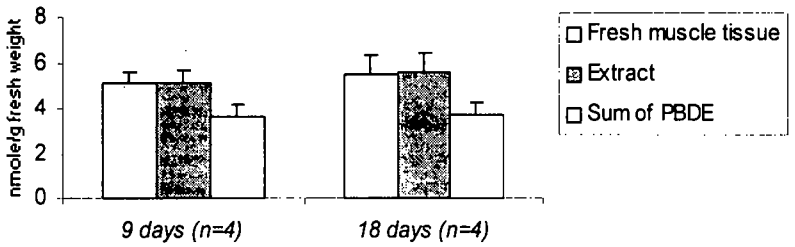

Figure 1. Mean concentrations of ^{14}C in fresh muscle tissues, of ^{14}C in muscle extracts and of the sum of BDE28, BDE47 and BDE99 in the extracts quantified with GC/MS. Vertical bars represent standard errors.

2,4,4'-tribromo- and 2,2',4,4',5-pentabromodiphenyl ether (BDE28, BDE99) together with a few unidentified brominated compounds were present as minor contaminants (< 2%) in the synthesized BDE47 product used [9]. The sum of BDE47, -28 and BDE99, in the extracts as determined by GC/MS comprised on average 70% of the radioactivity in the same extracts (Fig. 1). The observed difference suggests the presence of metabolites. Fresh muscle tissue and extracts had about the same levels of radioactivity, indicating that no or just traces of water soluble and non-extractable products were formed.

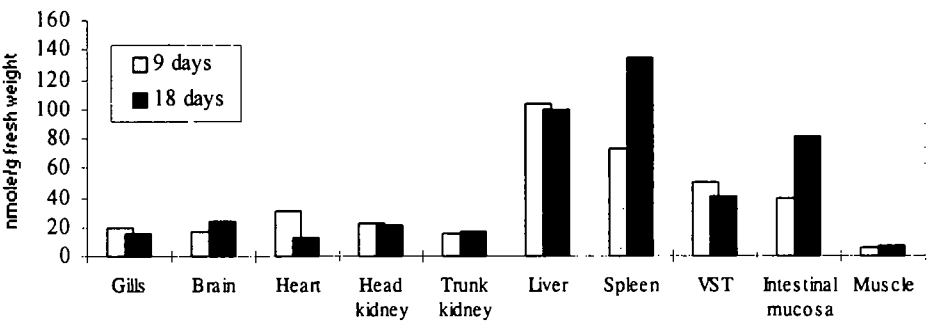

Figure 2. Distribution of ^{14}C in various extracts of pooled organ/tissues of pike expressed as nmole/g fresh weight after 9 and 18 days, respectively.

The distribution of extractable ^{14}C in sampled tissues is presented in figure 2. The highest levels were present in liver, spleen, intestinal mucosa and the lipid rich tissue surrounding vertebrae (VST). The levels in the pooled samples at 18 days exceeded those from 9 days in spleen, and intestinal mucosa, while the levels in heart were lower at 18 days. With the exception of the brain, the tissues with the highest lipid content also had high levels of extractable ^{14}C, i.e. on lipid weight basis the distribution was more even. The distribution pattern is principally in accordance to the earlier autoradiography study except for the spleen, which in the former study had low levels [12]. The authors concluded that the radioactivity was accumulated in lipid rich tissues where only small or no decline was seen after 65 days.

In the present study more than 95% of the radioactivity in organ/tissues was extractable. Only minor amounts were non-extractable (< 2%) or found in the lipid fraction (< 1%). Little or no water-soluble ^{14}C was detected (< 1%).

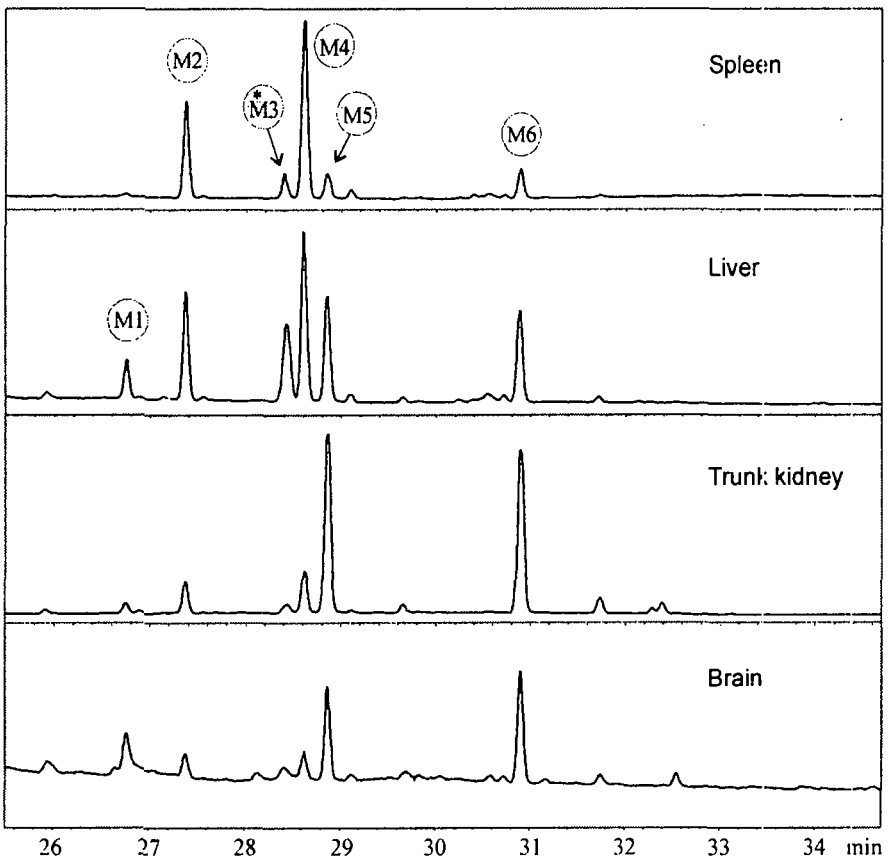

Figure 3. Mass chromatogram (ECNI, m/z $79^- + 81^-$) of methylated phenol fractions from different organs in pike (* at least two coeluting compounds).

After methylation of the phenol fraction a number of unidentified peaks were observed. Mass chromatograms of the late eluting compounds (here presented as M1 to M6) are presented in Figure 3. These peaks were present in all tissues analysed although at varying levels. Five mono-

hydroxylated tetraBDEs were earlier indicated in rat and mice after oral administration of ^{14}C-labelled BDE47 [3]. Due to the symmetry of the parent compound, there are six possible mono-hydroxylated metabolites of BDE47, either through direct hydroxylation or via *1,2*-shift i.e. metabolism via an arene oxide. In the rat/mice study two *ortho*-, one *meta*- and two *para*-substituted OH-tetraBDEs, in order of elution on a GC column, were indicated. In the present study M2 matched the retention time of 2-methoxy-2',4,4',6-tetrabromodiphenyl ether and 2-methoxy-2',3,4',4-tetrabromodiphenyl ether corresponded to the retention time of M3.

The profile varied between different organs (Fig. 3). Spleen, liver, vertebrae surrounding tissue were dominated by M4 and M2, whereas the profile of trunk kidney, brain and to some extent the gills were dominated by peak M5 and M6. Selective retention has earlier been reported for hydroxylated PCB metabolites, especially *para*-substituted, in blood and brain tissue [13].

Reductive debromination as a metabolic pathway was earlier indicated in rainbow trout exposed to decabromodiphenyl ether via the diet [14]. In the present study there were no significant signs of debromination although the issue is complicated by the fact that the synthesized BDE47 product used in the experiment also contained traces of lower brominated PBDEs that may be identical to those metabolically produced.

Further studies are in progress aiming to characterize/identify metabolites in blood and bile.

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