UPTAKE IN ZEBRAFISH OF A STRUCTURALLY DIVERSE SET OF BROMINATED FLAME RETARDANTS AFTER DIETARY EXPOSURE

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Introduction

Brominated flame retardants (BFRs) are a structurally diverse group, including aliphatic, cycloaliphatic, and aromatic compounds. A set of ten BFRs has previously been reported and suggested to be used for screening of BFRs persistency, bioaccumulation and toxicity (PBT) properties[1]. These ten BFRs were selected as representatives for the group of brominated flame retardants to cover their variation in molecular structure and physicochemical properties. The ten selected BFRs include from the highly brominated decabromodiphenyl ether to the monobrominated compound 2-Bromostyrene. The aim of this study was to investigate the uptake in male zebrafish of structurally diverse BFRs during dietary exposure. An eleventh compound, tetrabromobisphenol A 2,3-dibromopropyl ether, was added to the set as reported by Andersson et al[1]. The molecular structures, abbreviations, chemical names and CAS registry numbers for the eleven compounds included in this study are presented in Figure 1.

The accumulation after dietary exposure can be described by the assimilation efficiency, i.e. the fraction of the consumed compound that is stored in the fish, or by biomagnification factors (BMF), i.e. the concentration measured in the fish at steady-state divided with the concentration in the feed[2]. The BMFs in zebrafish for BDE 28 and BDE 47 have been reported to be >1, and <0.5 for BDEs 66, 85, 99, 100, 138, 153 and 154 [3]. The assimilation efficiency in salmon for several PBDEs has been reported by Isosaari et al[2]. For juvenile carp no accumulation was observed after 60 days of dietary exposure of BDE 209[4]. However, these authors reported that seven penta to octa brominated metabolites of BDE 209 were accumulating in the fish.

Materials and Methods

Zebrafishes were fed with freeze-dried chironomids spiked with BFRs at about 2% of their body weight. The sampling was carried out after 0, 3, 7, 14, 28, 35 and 42 days. After the exposure period sampling was carried out after 7 and 14 days to study the elimination of the compounds. In parallel endocrine effects of the BFR mixture were studied, which however will not be discussed in this report.

The chironomid feed was prepared by adding a mixture of the eleven selected BFRs dissolved in ethanol to the chironomids to a nominal concentration of 100 nmol/g dry weight. 2,4,6BrPh, HxBrBz, BrCyHx, TBBPA OHEE, 2BrSty and HBCD were purchased from Sigma-Aldrich, BDE 28, BDE 183 and BDE 209 from Stockholm University, TBBPA from Promochem and TBBPA DBPE from Labora AB (for abbreviations see Figure 1). After mixing with a stainless steel spoon the ethanol was allowed to evaporate. The sampled fishes were stored in a freezer (-20ºC) until the chemical analysis. From each sampling occasion, two fishes were pooled and homogenized in sodium sulfate impregnated with one percent sulfuric acid according to the method described by Berger et al[5]. Internal standards (^{13}C-labeled BDE 77, TBBPA and PCB 194) were added and the extraction was performed on an open column with acetone:hexane (5:2) and hexane:diethyl ether (9:1). The lipid weight was determined gravimetrically. For the chironomids, 1 g was homogenized and after lipid determination the sample was splitted. One tens of the sample was spiked with the internal standards. The lipids were removed with gel permeation chromatography (GPC) using SX-3 Bio-beads in cyclohexane:ethyl acetate (3:1). The flow rate was set to 5 ml/min and the BFRs were sampled between 18 and 45 minutes. The sample was further purified and fractionated on a

florisil column, where the first fraction, including the PBDEs, HBCD, BrCyHx, HxBrBz, TBBPA DBPE, and BrSty, was eluted with hexane:dichloromethane (3:1) and the second fraction, including BrPh, TBBPA, and TBBPA OHEE, was eluted with dichloromethane:methanol (88:12). Traces of fat were removed from the first fraction on a mini acid silica column and the analysis was done on an Agilent GC-LRMS (MSD 5975) in ECNI mode. The analytes in the second fraction were derivatized with BSTFA, forming trimethyl silyl derivatives, prior to injection on GC-MS in EI mode. All analyses were performed with a 15 m × 0.25 mm i.d. (0.10μm film thickness) DB-5MS column and ions were recorded in SIM mode. The biomagnification factors were calculated by dividing the concentration in the fish by the concentration in the chironomids, both on lipid basis.

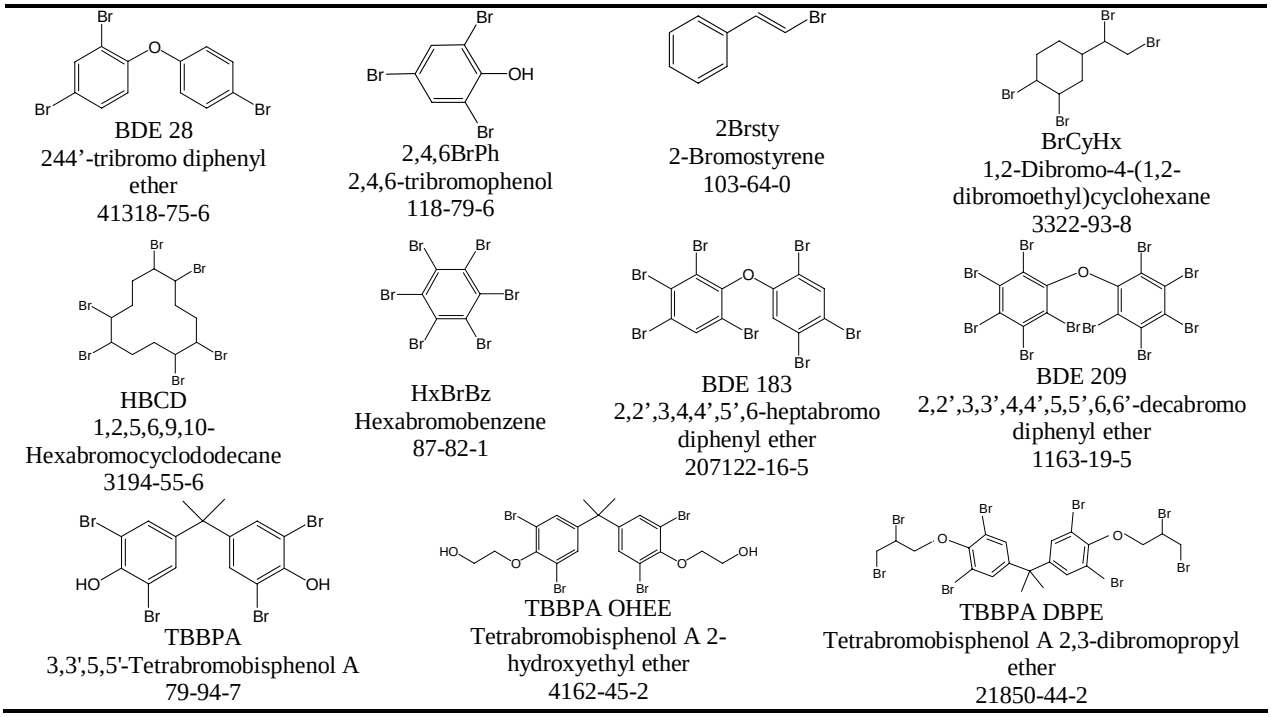

Figure 1. Structures, abbreviations, chemical names and CAS registry numbers for the chemicals included in this study.

Results and Discussion

Eight of the eleven studied BFRs were identified in all samples and only TBBPA DBPE and 2BrSty could not be detected in any fish sample. TBBPA OHEE was only detected in the day 3 and day 7 samples. The concentrations of BBDE 28, HBCD, BDE 183, and BrCyHx in zebrafish during the 42 days exposure study are shown in Figure 2 The amounts of BDE 209, HxBrBz, 2,4,6BrPh, and TBBPA in the fishes were low and are not shown in the figure. No or only small increase in concentration can be seen after three days of exposure. The low concentrations for BDE 28 and HBCD in day 14 might be explained by individual differences or analytical problems. BMF values were calculated using the concentrations determined after 42 days of exposure, although steady-state conditions for any of the assessed chemicals could not be defined (Figure 3).

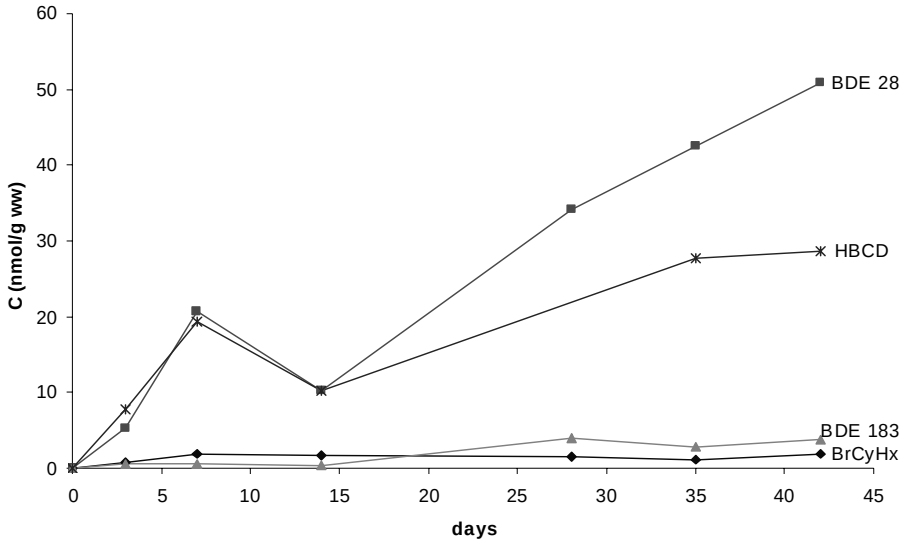

Figure 2. Concentrations of BDE 28, HBCD, BDE 183, and BrCyHx in male zebrafish after dietary exposure.

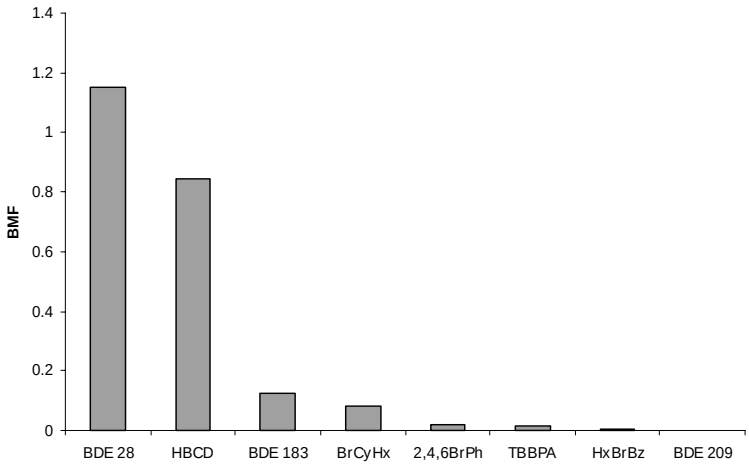

Figure 3. Biomagnification factors for BFRs in male zebrafish exposed for 42 days.

The eight BFRs that were detected in the sample taken at day 42 were also present after the fourteen days long elimination period, but in lower concentrations. The elimination appears to be fast for BrCyHx and slower for HBCD and BDE 28. The low levels of the majority of the brominated compounds included in this study suggest that these compounds have low bioavailability or were rapidly excreted or metabolized. In Figure 4, chromatograms for spiked chironomids and fishes exposed for 42 days can be seen. There are several peaks in the fish sample that are not present in the chironomids, which probably are metabolites or degradation products. Peaks marked in Figure 4 can, based on their EI full scan spectra, be suggested to be an unidentified tribrominated compound (1), a tetrabromobenzene (2), and a tetra- (3), penta- (4), and hexa-BDEs (5,6,7). These are potential breakdown products

from BDE 183 or BDE 209 and HxBrBz. Further, these compounds were increasing during the exposure period and were still present after the fourteen days elimination period.

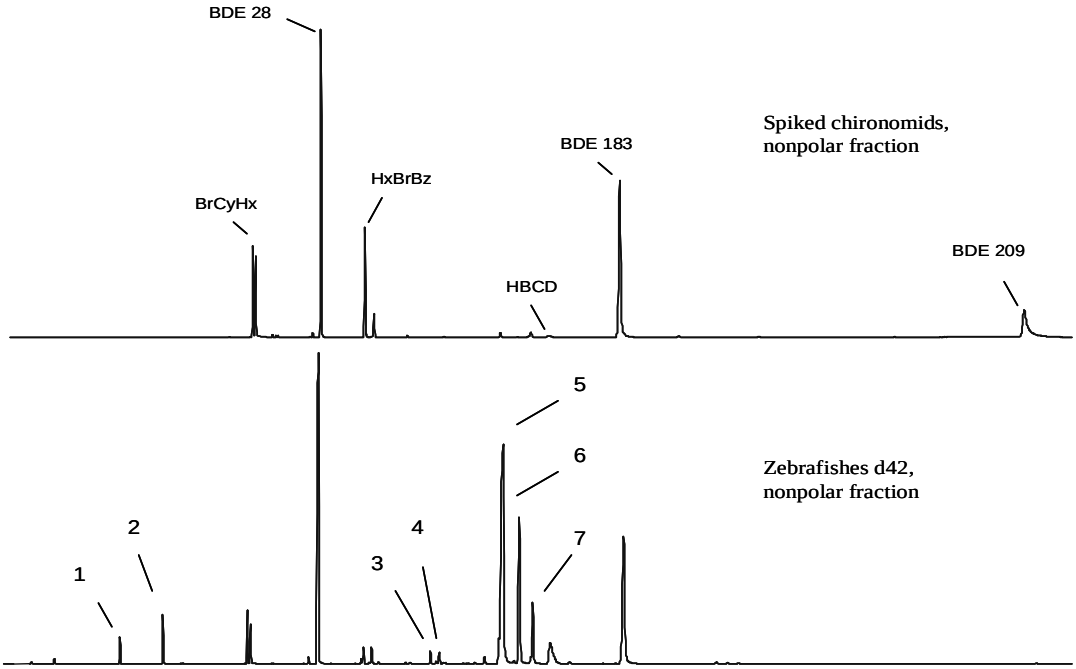

Figure 4. Chromatograms for the nonpolar fraction for the chironomids and the zebrafish sampled at day 42. The bromide ion m/z 79 was monitored in ECNI-MS.

In conclusion, a high uptake could only be seen for two compounds, viz. BDE 28 and HBCD. The calculated BMF for BDE 28 is very close to the BMF reported by Andersson et al. in a similar study[3]. For hydroxylated and highly brominated compounds the concentrations in the fishes were very low compared to the concentration in the feed. A number of yet unidentified metabolites or degradation products were observed in the fish. Analysis of these compounds, the diastereoisomers of HBCD plus additional exposure levels will be carried out in the near future.

Acknowledgements
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