

Maternal Transfer in Zebrafish Exposed to a Structurally Diverse Set of Brominated Flame Retardants

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Introduction

Brominated flame retardants (BFRs) are chemicals used for inhibition of fire. Some of the BFRs may pose a threat to man and the environment since these chemicals are persistent and bioaccumulate in biota. In this study adult zebrafish were exposed to a mixture of structurally diverse brominated flame retardants. The objective was to study the uptake in the adults and the transfer to the offspring. Eleven BFRs were included in the mixture (Figure 1), ten of them were taken from a set that has previously been reported and suggested to be used for screening the persistency, bioaccumulation potential and toxicity (PBT) of BFRs (Andersson et al. 2006). In parallel to the accumulation study, effects on reproduction after dietary exposure were studied, showing an increase in atretic oocytes in female fishes and reduced hatching success (Norman et al. 2007).

Maternal transfer of several BFRs has previously been observed for e.g. birds. For example HBCD, BDEs 28, 47, 99, 100, 153, 154, and 183 have been detected in eggs from little owl (Jaspers et al. 2005), and TBBPA in eggs from birds of prey (Berger et al. 2004). After exposure in ovo to a mixture of BDEs 47, 99, 100, 153 and 154, changes in growth in American kestrel nestlings has been reported (Ferne et al. 2006).

Materials and Methods

Zebrafish were dietary exposed to eleven structurally diverse brominated flame retardants (see Figure 1). The fish were fed with freeze-dried chironomides at 2% of their body weight per day for 42 days, spiked at the nominal concentration of 100 nmol/g. During the exposure period fish were sacrificed after 0, 3, 7, 14, 28, 35, and 42 days, and eggs were collected and pooled after 0, 2-3, 6-7, 13-14, 27-28, 34-36, and 41-42 days. The fish and egg samples were stored in the freezer (-20°C) 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 2004. For the egg samples, 0.78-3.8g wet weight was used. Internal standards, ¹³C-labeled BDE 77, PCB 194, TBBPA, and BDE 209, were added and the extraction was performed on an open column with 60 mL acetone:hexane (5:2) and 60 mL hexane:diethyl ether (9:1). The lipid weight was determined gravimetrically. The lipids were removed with gel permeation chromatography (GPC) using SX-3 Bio-beads in cyclohexane:ethyl acetate (3:1). After GPC the sample was 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 40% H₂SO₄-silica column and analyzed on GC-MS in both EI and CI 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. (0.10 μm film thickness) DB-5MS column (J&W Scientific, Folsom, CA, USA) and ions were recorded in SIM mode.

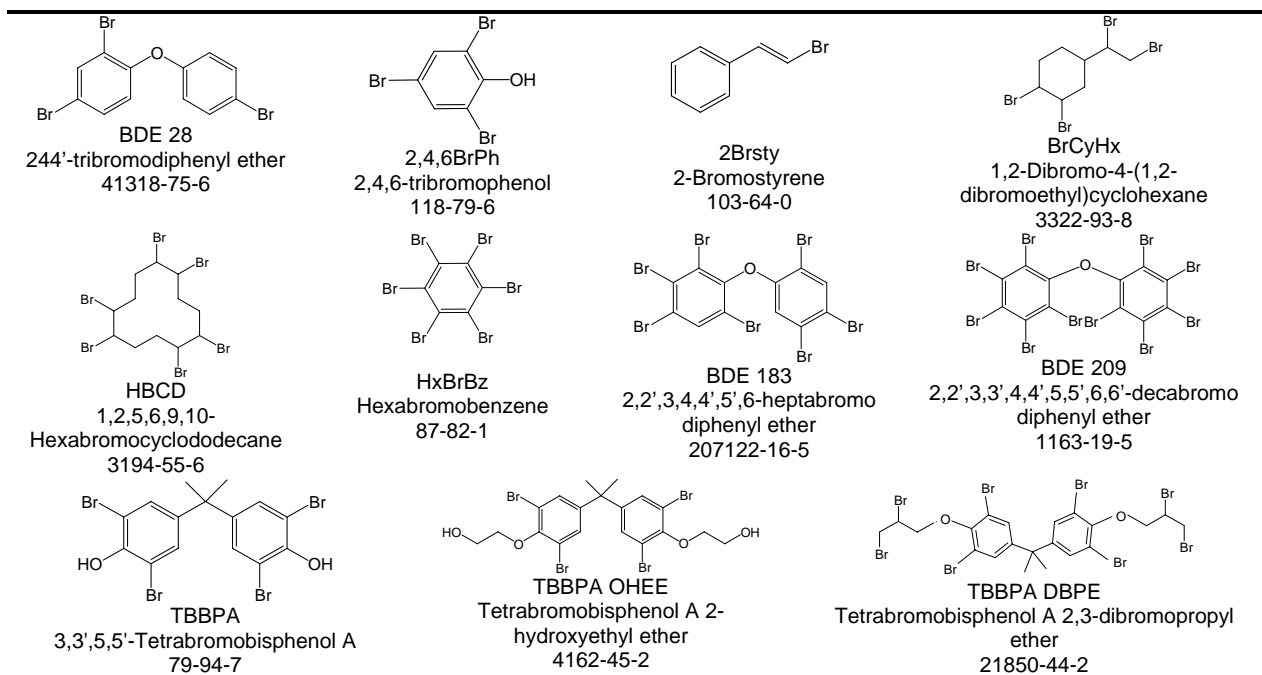


Figure 1. Structures, abbreviations, chemical names and CAS registry numbers for the chemicals included in this study.

Results and Discussion

In female zebrafish, the levels of BDE 28, HBCD, BDE 183, BrCyHx, and BDE 209 were quantified. The levels for BDE 28, HBCD, BDE 183, and BrCyHx can be seen in Figure 2, and the level of BDE 209 was 0.13 nmol/g ww after 42 days exposure. 2BrSty, HxBrBz, TBBPA DBPE, 2,4,6BrPh, TBBPA, and TBBPA OHEE were not detected or found under the limit of quantification (LOQ) (three times above the levels in the laboratory blank). 2,4,6BrPh and TBBPA were probably not detected because of analytical problems resulting in very low recoveries of these compounds. A chromatogram from a nonpolar fraction can be seen in Figure 3, showing a number of additional peaks that are probably metabolites or degradation products of the BFRs. The three largest peaks have, based on full scan spectra in EI mode, been determined to be hexabromodiphenyl ethers.

In the eggs, BDE 28, HBCD, BDE 183, BrCyHx, 2,4,6BrPh, TBBPA, and BDE 209 were quantified. The levels of BDE 28, HBCD, BDE 183, and BrCyHx in eggs can be seen in Figure 4. 2BrSty, HxBrBz, TBBPA DBPE, and TBBPA OHEE were not detected or under the LOQ. The levels of BDEs 28 and 183 were approximately ten respectively five times lower in the eggs than in the female fish based on wet

weight. The largest peaks of the metabolites/ degradation products found in the female fish were also found in the eggs (Figure 3).

In conclusion, a maternal transfer to zebrafish eggs can be seen for all BFRs detected in the female zebrafish. In the near future we will study the identity of the hexabromodiphenyl ethers and other metabolites/ degradation products found in the exposed fish and the eggs.

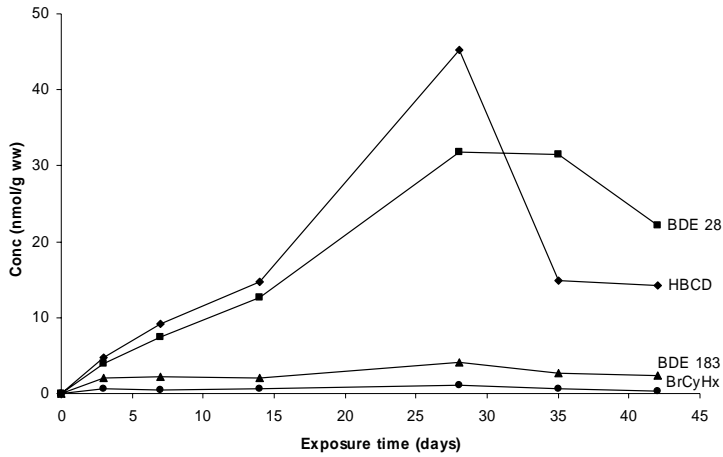


Figure 2. Levels in female fish during the exposure period.

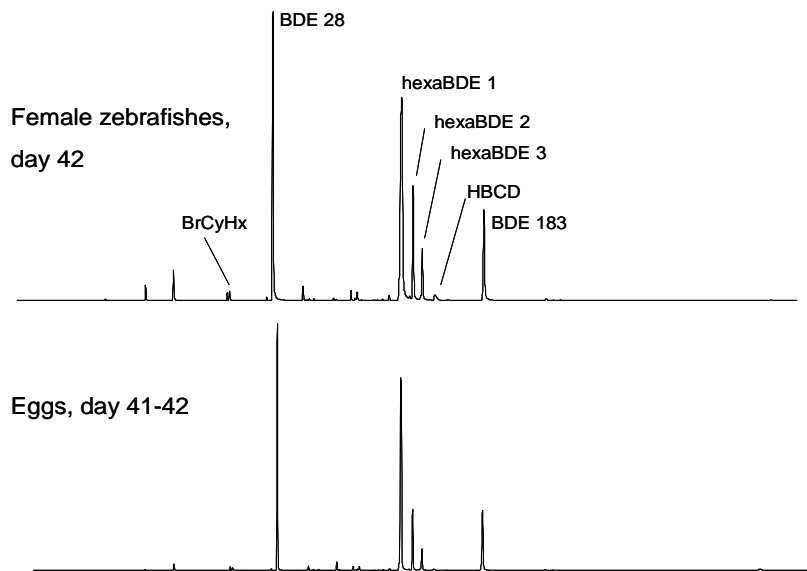


Figure 3. ECNI-MS chromatograms for the nonpolar fraction for female zebrafish sampled after 42 days of dietary exposure and eggs sampled after 41-42 days.

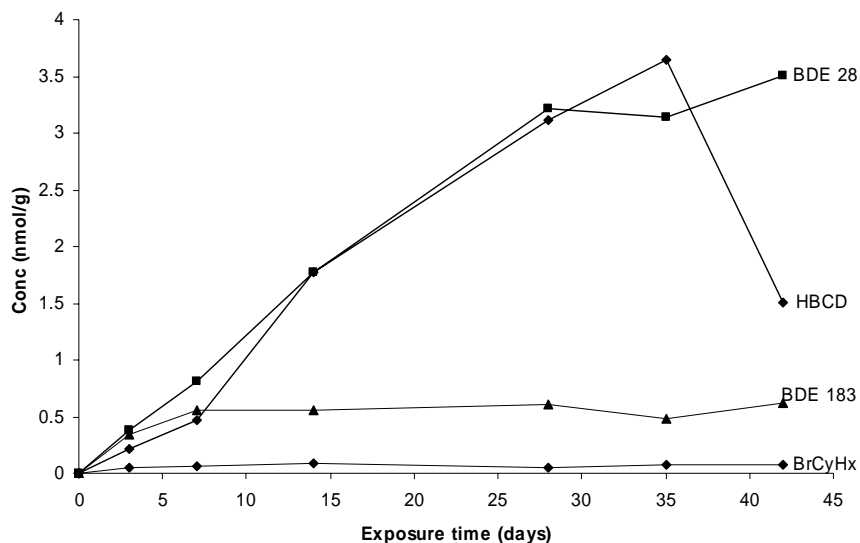


Figure 4. Levels in the eggs collected during the exposure period.

Acknowledgements

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References

- Andersson PL, Öberg K, Örn U. 2006. *Environmental Toxicology and Chemistry* 25:1275
- Berger U, Herzke D, Sandanger TM. 2004. *Analytical Chemistry* 76:441
- Fernie K, Shutt L, Ritchie I, Letcher R, Drouillard K, Bird D. 2006. *Journal of Toxicology and Environmental Health* 69:1541
- Jaspers, Covaci A, Maervoet J, Dauvwe T, Voorspoels S, Schepens P, Eens M. 2005. *Environmental Pollution* 136:81
- Norman A, Rattfelt J, Andersson PL, Norrgren L. 2007. *BFR 2007*, Amsterdam, The Netherlands