Reproduction effects in zebrafish exposed to a mixture of structurally diverse brominated flame retardants

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Introduction

Brominated flame retardants (BFRs) are used in many materials to prevent fire. They are spread into the environment from production, use and disposal and have been detected in humans as well as in wildlife. The highest levels in wildlife are often found in the aquatic environment. The BFRs are a structurally diverse group with varying chemical properties. A set of BFRs, selected to cover the variation in physicochemical properties, has previously been suggested for screening of BFRs persistency, bioaccumulation and toxicity (PBT) properties (Andersson et al. 2006). In the present study, a mixture of these selected BFRs and an additional 11th compound (Figure 1), was given to adult zebrafish in order to study effects on reproduction. In a parallel study, uptake of the compounds in adult zebrafish and transfer to offspring were studied (Rattfelt et al. 2007).

Materials and Methods

The BFR mixture (1, 10, 100 nmol/g) and ethanol only (solvent control) was added to freeze-dried chironomids and given to the fish in amounts equivalent to 2% of their body weight per day. As a complement, fish were fed with artemia. Throughout the experiment, fish and eggs were kept in aerated standardised water (ISO7346-1 1996) at a temperature of 26°C. The photoperiod was set to 12h. The experimental groups consisted of 12 adult zebrafish (approximately 8 males and 4 females) and were set in duplicates. Fecundity, spawning success (number of days with successful spawning) and fertilisation success were studied during a pre-exposure period of 21 days followed by 21 days of exposure. At the end of the exposure period, fish were sampled and body, gonad and liver weights were measured. Hepatic and gonad somatic index were calculated and female and male gonad histology was studied. Hatching success was studied in the offspring at the end of the pre-exposure and the exposure period, respectively. Newly fertilised eggs were randomly selected from each replicate and divided to duplicate petri dishes with standardised water. The number of dead eggs and larvae were counted two times per day.

Fecundity and fertilisation success were compared within groups between the pre-exposure and the exposure period using a two-way ANOVA. A one-way ANOVA was used to test differences in GSI and HSI. Fisher´s Exact Test was used to test differences in spawning success within groups between the pre-exposure and the exposure period. The data on hatching success was compared using Fisher´s Exact Test with a following Bonferroni adjustment. Differences in number of atretic oocytes was analysed using the non-parametric Mann-Whitney U-test with a following Bonferroni adjustment. The significance level was set at 95% ($p<0.05$).

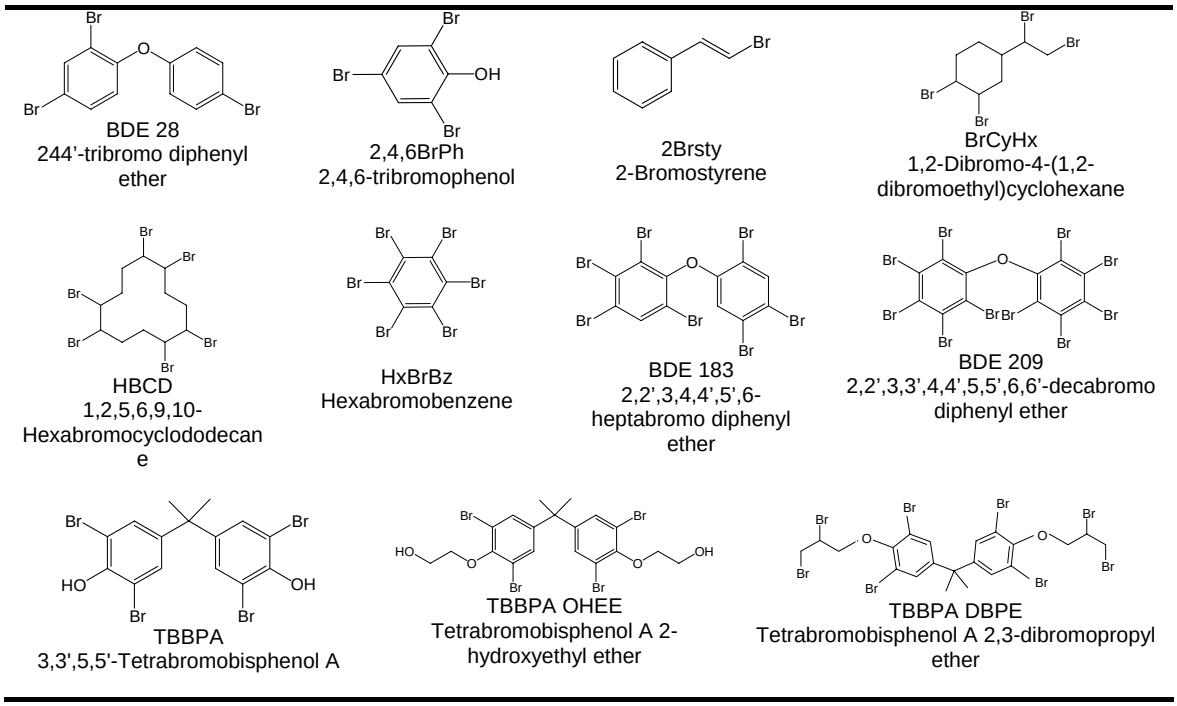

Figure 1. Structures, abbreviations and chemical names for the chemicals included in this study.

Results and Discussion

Histological examination of the ovaries revealed a dose related increase in number of atretic oocytes (Figure 2). No difference was seen in gonad somatic index, hepatosomatic index or male gonad morphology. The fertilisation success was over 75% in all groups throughout the experiment and was not affected by the different treatments.

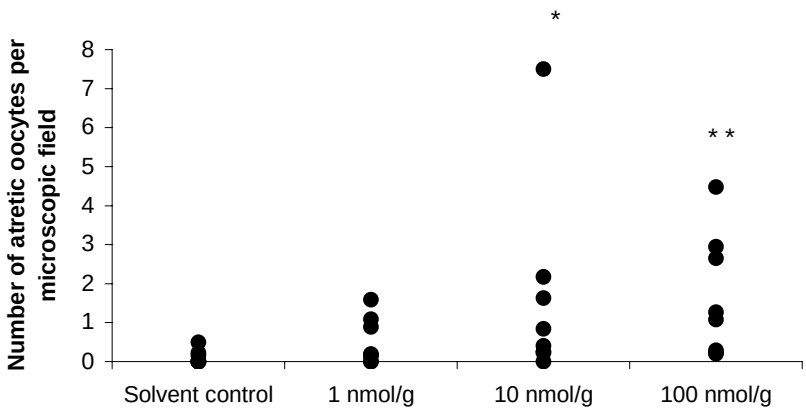

Figure 2. Number of atretic oocytes in ovaries from female zebrafish dietary exposed to 0, 1, 10, or 100 nmol BFR/g for 21 days. * and ** denotes significant ($p<0.05$ and $p<0.01$, respectively) difference from the solvent control.

No exposure related difference was recorded in fecundity but a significantly lowered spawning success was found in the high dose group (Figure 3).

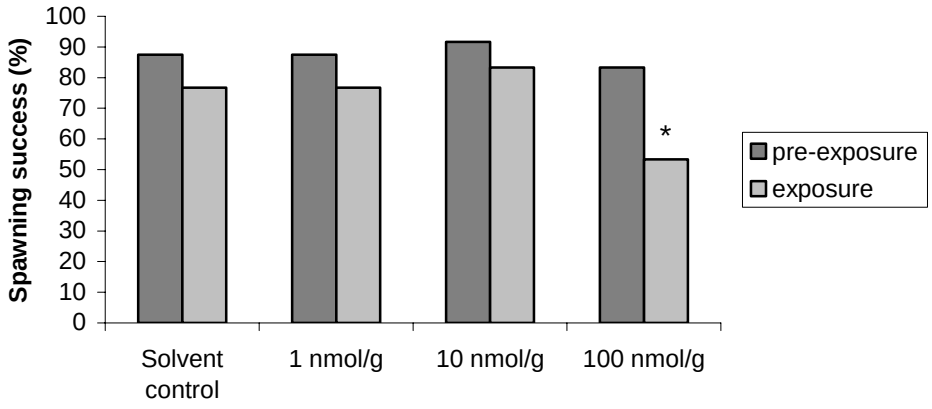

Figure 3. Spawning success (%) in zebrafish fed uncontaminated food for 21 days (pre-exposure) and food spiked with 0, 1, 10, or 100 nmol BFR/g the following 21 days (exposure).* denotes significant ($p<0.05$) difference between the pre-exposure and the exposure period.

A decreased hatching success was observed in the high dose group compared to the control (Figure 4). In the parallel uptake study, BDE 28, HBCD, BDE 183, BrCyHx, 2,4,6BrPh, TBBPA and BDE 209 were detected in eggs from exposed parental fish (Rattfelt et al. 2007).

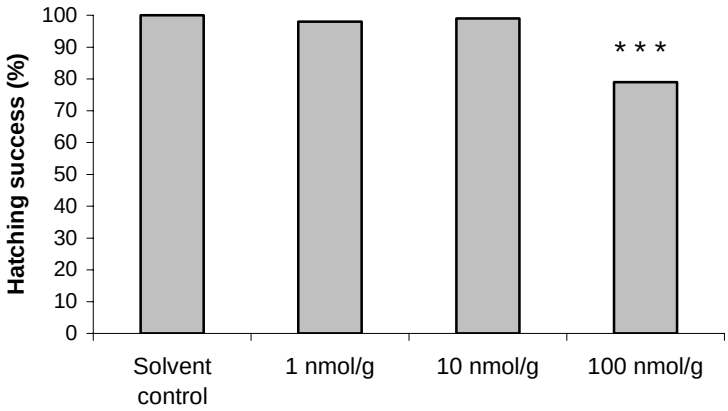

Figure 4. Hatching success (%) in offspring from zebrafish dietary exposed to 0, 1, 10, or 100 nmol BFR/g for 21 days. *** denotes significant ($p<0.001$) difference from the solvent control.

In conclusion, a dose related increase in number of atretic oocytes was seen in female fish exposed to the BFR mixture, which might indicate disturbed ovulation. Exposure to the BFR high dose resulted in a lowered spawning success. A reduced hatching success was seen in offspring from fish exposed to

the BFR high dose. Uptake in adult fish and maternal transfer was shown for the BFR mixture in a parallel study (Rattfelt et al. 2007).

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