Effects of PBDE-47 on thyroid and steroid hormone status in juvenile turbot (Schophtalamus maximus)

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Introduction

Many of the brominated flame retardant (BFR) chemicals, and particularly polybrominated diphenyl ethers (PBDEs), has become of increasing concern to scientists over the past decade. Many of the PBDEs are persistent and lipophilic and have been shown to bioaccumulate. The levels of PBDEs in biota seem to be increasing, and several trends, including in humans, indicate that this increase may be rapid[1]. In general, BFRs have a low acute toxicity, but there is concern about their long-term toxic effects. Exposure studies have revealed a range of subtle biochemical, cellular and physiological effects following low-dose exposure, and many BFRs have been reported to have endocrine disruptive properties. Thus, there is concern about their potential to affect organisms and populations.

The mechanisms through which endocrine disrupters (EDs) can affect the reproductive health and survival of wildlife are diverse. Estrogens and their mimics are present in marine waters at concentrations that can cause negative biological responses in fish. Disruptions of steroid hormones, such as estradiol (E) and testosterone (T), might affect the reproduction, and may thus have a direct effect on Darwinian fitness. The most extreme manifestations are feminization phenomena with spermatogenesis reduction and apparition of oocytes in gonads of some male fish species. The presence of the female yolk protein vitellogenin in male fish has also been commonly reported in feral populations of fish[2].

Thyroid hormones (THs) play an important role in organism's development, metabolism, growth and behavior[3]. Polyhalogenated aromatic hydrocarbons (PHAHs) including BFRs may affect the thyroid system through several mechanisms. They may directly affect the thyroid gland function, the peripheral metabolism of THs and/or the binding of THs to plasma transport proteins[4]. Effects of PHAHs on TH homeostasis have been documented in a number of species, including fish[5].

Du to its persistence against degradation PBDE-47 is among the most abundant PBDE congener in biota[1], and there is a great concern about its ecotoxicological effects on organisms and populations.

The aim of the present study was to examine if PBDE-47 may affect levels of circulating steroid and thyroid hormones in juvenile turbot (*Scophtalamus maximus*). The turbot is a benthic living

flatfish that can be exposed to PHAHs via the sediment living organisms. Thus, plasma levels of T, E, and the thyroid hormones thyroxine (T4) and triiodothyronine (T3) were determined in juvenile turbot that had been continuously exposed to PBDE-47 via water for 3 weeks.

Materials and methods

The exposure studies were conducted at Akvamiljø, Rogalandforskning AS, Stavanger, Norway in April 2003. Juvenile turbots of both sexes were randomly divided into experimental groups and kept in separate tanks with continuous seawater flow-through. One group was exposed to a constant water concentration of 5 ppb of PBDE-47 for three weeks whereas one group served as the control group. Except for the exposure to the chemical, the two groups were treated identically. The seawater temperature in the tanks was 11 °C, and the fish were fed daily (Dana feed pellets, 5 mm), but were fasted the last week prior to sampling. Following the three week experimental period, a heparinised blood sample was taken from the fish. The blood sample was centrifuged and the plasma was frozen for later analyses of hormones.

The concentrations of T and E (17 β-estradiol) were analyzed in 16 male fishes from the control group, and in 12 male fish from the PBDE-47 exposed group. Plasma concentrations of total T3 and total T4 were analysed in 20 female fish from the control group and in 21 female fish from the PBDE-47 exposed group. All hormones were determined by Radioimmunoassay (RIA) using commercially available kits (Spectria Testosterone [^{125}I] and Spectria Estadiol [^{125}I] sensitive, Orion Diagnostica, Finland; total T3 and total T4 COAT-A-COUNT® DPC®, Diagnostic Products Corporation, Los Angeles, USA). Because of low plasma concentrations of T4, the standards were diluted with a factor of 2 prior to analysis.

Statistical analysis was conducted using SPSS statistical software version 12 (SPSS, Chicago, IL, USA). Shapiro-Wilk test was used to test normality, and T, T4 and T3 concentrations were $\log^{10}$-transformed to achieve normality. Homogeneity of variance was tested using Levene's test, and Student's *t*-test was used to test differences in plasma concentrations between control and exposed fish. Plasma concentrations of E deviated significantly from normality even after $\log^{10}$-transformation. Mann-Whitney U-test was therefore used to test the difference in E between the two groups. The level of significance was defined as $P < 0.05$.

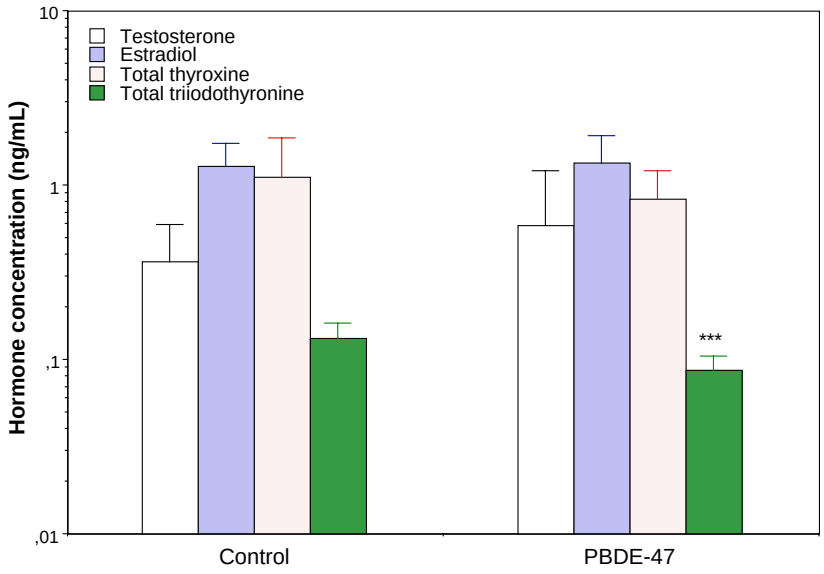
Results and discussion

There were no significant differences between the control group and the PBDE-47 exposed group of juvenile turbots with respect to size (length) or body mass. As shown in Fig. 1, the plasma concentration of T3 was significantly lower in the turbots that were exposed to PBDE-47 as compared to the control group ($t = 5.42$, $df = 40$, $P < 0.001$).

There were no significant differences in the plasma concentrations of T, E and T4 between the control and the PBDE-47 exposed group ($P = 0.61$, 0.12 and 0.27 respectively).

In a recent study, lowered plasma levels of free T4 was reported in juvenile lake trout (*Salvelinus namaycush*) experimentally exposed to 13 PBDE congeners via their diet (0, 0.25, 25 µg/g per PBDE congener)[6]. In the present study on juvenile turbot, no effect was documented on levels of total plasma T4. It should be noted that in the present study on turbots, plasma concentrations of total T4 were measured, whereas in the study on lake trout, free T4 was measured[6]. Thus, it is possible that the differing effects of PBDEs on T4 in these two studies may be due to that the free and the total fractions of T4 in fish plasma are differently affected by PBDEs. The diverging results on T4 in the two studies may also be due to that the T4 depressing effects observed in PBDE exposed juvenile lake trout not was caused by PBDE-47, but by one or several of the other 12 PBDE congeners in the fish feed, or their metabolites. The differing effects may of course also be related to differing exposure routes or doses. Furthermore, the differing effects may be due to age or species specific differences in the effects of PBDEs.

In the organism T3 is the active hormone of the two THs (T3 and T4), and it is therefore possible that the apparent T3 depressing effect of PBDE-47 is an effect of functional importance in juvenile turbot. However, it should be noted that it is the free T3 that has the highest affinity to the TH-receptor. Due to the low plasma volumes obtained from the turbots, free T3 could not be analysed. However, a seemingly transient effect of PBDEs on free T3 has been reported in juvenile lake trout[6], and this may indicate that T3 is a relevant variable to study in order to document functional effects of PBDEs on thyroid function. The importance of including plasma concentrations of both free and total T3 and T4 have been demonstrated in a study of relationships between PCBs and THs in polar bears[7].



*** indicates a significant difference (P<0.001) between the control and the exposed group.

Figure 1. Concentrations (mean ± standard deviation) of testosterone, estradiol, total thyroxin and total triiodothyronine in juvenile turbot (*Scophtalamus maximus*) exposed to a water concentration of 5 ppm PBDE-47 for 3 weeks.

In the present study, no differences in plasma concentrations of testosterone and estradiol were found between males in the control group and males in the group exposed to PBDE-47. In male juvenile Atlantic salmon (*Salmo salar*) exposure to commercial mixtures of Penta-BDE and Octa-PDEs has been demonstrated to have no effect on vitellogenin or zona radiata proteins[8]. Together with the lack of effect of PBDE-47 on plasma estrogen and testosterone concentrations reported in the juvenile male turbots herein, this indicates that PBDEs have a low potency of causing estrogenic hormone related effects in juvenile male fish. However, it should be noted that it has been reported that female fish may be more susceptible to steroid hormone disruption than male fish[9]. Thus, we recommend that future studies concerning effects of BFRs (and other PHAHs) on hormone status and function of fish consider possible inter-sex differences in effects.

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