ENDOCRINE DISRUPTING AND BIOCHEMICAL EFFECTS OF PBDES IN A SONGBIRD, THE EUROPEAN STARLING (*STURNUS VULGARIS*)

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Abstract
We exposed female European starlings (*Sturnus vulgaris*) to environmentally relevant concentrations of a pentabromodiphenyl ether (Penta-BDE) mixture through subcutaneous implants, and examined endocrine disruptive and biochemical effects. Sum PBDEs accumulated in the serum of the exposed females from 218 ± 43 pg/ml to a peak concentration of 23 400 ± 2030 pg/ml. Sum PBDE concentrations in the control group ranged from 151 ± 27 pg/ml serum to 313 ± 132 pg/ml throughout the exposure period. To investigate endocrine disrupting effects of PBDEs, testosterone, estradiol and thyroid hormones (T3 and T4) were measured in the serum. No signficant differences were found between the control and exposed group in hormone concentrations. However, triiodothyronine (T3) concentrations were lower in the exposed group compared to the control group, although this difference was not significant. Our results also suggested that PBDEs may be responsible for lower albumin and triglyceride concentrations in the serum of exposed individuals. There were no significant differences found between the control and exposed group for the other biochemical parameters (total protein, uric acid, cholesterol, AST, total antioxidative capacity, HDL and creatine kinase). To our knowledge, our study is the first to report effects of PBDEs on albumin and triglyceride concentrations.

Introduction

Polybrominated biphenyl ethers (PBDEs) have been used on a large scale as flame retardants in several products, including textiles and electronics. Although the use of PBDEs has been banned, high concentrations still exist in the environment and little is known about the effects of these pollutants in humans and wildlife.

Potential health risks associated to PBDEs include endocrine disruption, neurotoxicity and teratogenicity[1,2]. However, only a limited number of studies have been performed on the adverse effects resulting from exposure to PBDEs in birds. Environmentally relevant concentrations of PBDEs have been shown to cause changes in growth, thyroid hormone and vitamin A concentrations, glutathione metabolism, oxidative stress and immune function in American kestrels (*Falco sparverius*)[3,4,5]. Female European starlings (*Sturnus vulgaris*) exposed to environmentally relevant concentrations of a Penta-BDE mixture initiated egg laying less frequently compared to the control group, although the difference was not significant[6].

In the present study, female European starlings were exposed to environmental levels of a Penta-BDE mixture using subcutaneous implants. The European starling is a terrestrial songbird species, which can be easily kept in captivity and which also shows normal reproductive and social behaviour in captivity[7], making it an ideal model to study effects after experimental exposure to PBDEs. To investigate endocrine disrupting effects of PBDEs, testosterone, estradiol and thyroid hormones were measured in the serum. In addition, several biochemical blood parameters were assessed and compared between the exposed and control group.

Materials and Methods

Silastic tubes (Degania silicone; length: 20 mm, inner diameter: 1.47 mm, outer diameter: 1.96 mm) were used to expose adult female starlings to an environmentally relevant concentration of PBDEs. The tubes were sealed at both sides with a medical adhesive. This implantation technique has previously been used to expose birds and fish to polychlorinated biphenyls (PCBs)[8-10]. Silastic implants have recently also been successfully used to expose European starlings to PCB 153 and BDE 209[11,12]. A technical Penta-BDE mixture (crystals, Dr. Ehrenstorfer GmbH) was dissolved in iso-octane and mixed in peanut oil (Sigma-Aldrich). The iso-octane was removed by gently heating (40 °C) the oil solution until constant weight. PBDE congeners 28, 47, 49, 66, 85, 99, 100, 153, 154 and 183 could be measured in the oil solutions. Sum PBDE concentration of the oil was 4978 ± 65 ng/µl (n = 6). Females of the exposure group (n = 15) received an implantation dose of about 150 µg and females of the control group (n = 14) received an implant filled with unfortified peanut oil.

After implantation, both experimental and control females were housed in the same aviary (L × W × H = 12 m × 8 m × 2.75 m). About 17 days after implantation[11,12], females were transferred to another large outdoor aviary (L × W × H = 24 m × 8 m × 2.75 m) in which 36 males were housed for almost one year. This aviary contained 29 wooden nest boxes mounted on the wall about 2 m above the ground. All nest boxes were identical and a 30 cm

wooden perch, attached to the bottom of each box, allowed the starlings to sit in front of it. At the moment the females were transferred, 23 males were occupying one or more nest boxes. Food and water were provided ad libitum. Each bird was marked with numbered metal and colour rings to allow individual identification. Regular observations were performed to assess pair formation and to identify which males and females occupied which nest boxes. Before the start of egg laying, nests were visited every 2-3 days depending on the nest building stage during the previous visit. Nest boxes were checked daily throughout the egg laying stage to determine the exact laying order of the entire clutch. Every freshly laid egg was carefully numbered with a permanent marker pen and replaced by a dummy egg, because intraspecific nest parasitism and egg removal has been reported to occur in European Starlings[13]. The oberved effects on reproductive parameters are reported in Van den Steen et al.[6].

Serum concentrations of PBDEs were monitored immediately before implantation (serum 1) and four times after implantation (serum 2: 5 days after implantation, serum 3: ~10 days after implantation, serum 4: ~17 days after implantation, serum 5: ~60 days after implantation) by taking blood samples (~300 µl). Blood samples were also taken before implantation (before), 10-14 days (after1) and about 2 months after implantation (after2) to investigate effects on biochemical parameters and hormones. To avoid disturbance of breeding activities, no blood samples were taken when the females were housed together with the males. Before each blood sampling, body mass was measured using a pesola balance. After collection, blood was centrifuged at 7000 rpm for 15 minutes and stored frozen until analysis.

In all samples, 10 PBDE congeners (BDE 28, 47, 49, 66, 85, 99, 100, 153, 154 and 183) were analysed. The following HO-PBDEs were also targeted: 2'-HO-BDE 68, 3-HO-BDE 47, 5-HO-BDE 47, 6-HO-BDE 47, 4-HO-BDE 42 and 4'-HO-BDE 49. PBDEs, an Agilent 6890 GC connected with an Agilent 5973 MS operated in electron capture negative ionisation (ECNI) mode was equipped with a 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). Limits of quantification (LOQs) for the analysed compounds ranged between 0.1 and 7.5 ng/g lw for the serum of the females.

For the biochemical parameters, all plasma samples were analysed with a Cobas MIRA S Plus Chemistry analyser (Horiba ABX, Montpellier, France). AST (aspartate transaminase), CK (creatine kinase), cholesterol, HDL (high-density lipoproteins), triglycerides, total protein, uric acid and albumin concentrations were measured using standard commercial kits (Horiba ABX, Montpellier, France). Repeatability (within-run precision) and reproducibility (run-to-run precision) of the measurements were certified by including a calibrator and a control (provided by Horiba ABX) with known concentrations each run and were in all cases between 95 and 105% of the certified value. As a measure for oxidative stress, we used the Trolox-equivalent antioxidant capacity measure of the plasma (TEAC). TEAC was measured as described in Erel (2004)[14] and the method was adapted for the Cobas MIRA S Plus Chemistry analyser[15]. This technique quantifies the total antioxidative capacity of the plasma by performing a reaction between the antioxidants in the plasma and a radical cation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), which results in a decolourization of the solution. The difference in absorption is measured spectrophotometrically at 660 nm, and compared to an internal standard (Trolox, a vitamin E analogue). The results are quantified as mM Trolox equivalents per liter.

Hormone concentrations were quantified in plasma extracts by radioimmunoassay (RIA). For testosterone and estradiol a commercial double antibody system (MP Biomedicals (Solon, Ohio)) was used. Antibodies used for the quantification of triiodothyronine (T3) and thyroxine (T4) were prepared in the laboratory of comparative endocrinology of the Catholic University of Leuven (Darras V.). The intra-assay coefficient of variation was 4.6 - 9.1 %.

Statistical calculations were performed using Statistica for Windows (Statsoft, 1997) on lipid-normalized concentrations. The level of significance was set at $\alpha = 0.05$ throughout this study. Before data analysis, samples with levels below LOQ were assigned a value of ½*LOQ. The data met the assumptions of normality and therefore parametric tests were performed. Repeated measures ANOVAs were performed to investigate potential effects of pentaBDEs on biochemical parameters and hormones. Post hoc tests were performed when there were significant effects.

Results and Discussion

Serum concentrations of PBDE

Sum PBDE concentrations in the control group ranged from 151 ± 27 pg/ml serum to 313 ± 132 pg/ml throughout the exposure period (Figure 1). Sum PBDEs accumulated in the serum of the exposed females from 218 ± 43 pg/ml to a peak concentration of 23 400 ± 2030 pg/ml before females and males were housed together (Figure 1). At the end of the experiment, sum PBDE concentrations had decreased to 17 200 ± 1610 pg/ml in the serum of the exposed starlings (Figure 1). These results have previously been described in Van den Steen et al. [6].

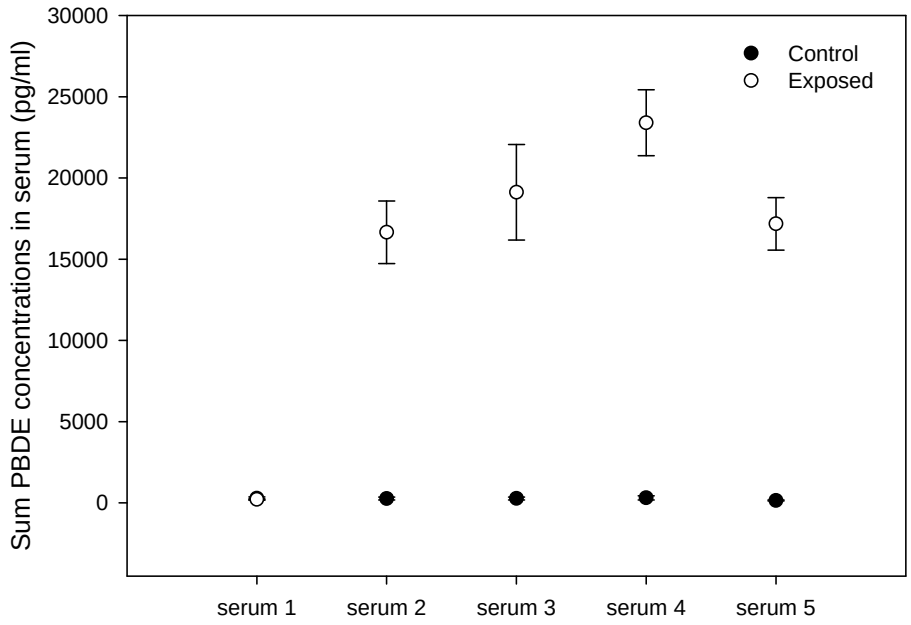

Figure 1: Sum PBDE concentrations in serum (pg/ml serum) of the female starlings from the control group (n = 14) and the exposed group (n = 15). Serum 1: before implantation, serum 2: 5 days after implantation, serum 3: ~10 days after implantation, serum 4: ~17 days after implantation (when females and males were housed together) and serum 5: ~60 days after implantation and after egg laying. Before implantation (serum 1), concentrations were similar in both groups.

Hormone concentrations

No significant differences were found between the control and exposed group in the hormone concentrations under study. However, T3 tended to be lower in exposed females compared to controls (Repeated measures ANOVA: treatment: $F_{1,20} = 3.63$, $p = 0.07$; time: $F_{2,42} = 4.13$, $p = 0.02$; treatment × time: $F_{2,42} = 0.72$, $p = 0.49$; Figure 2).

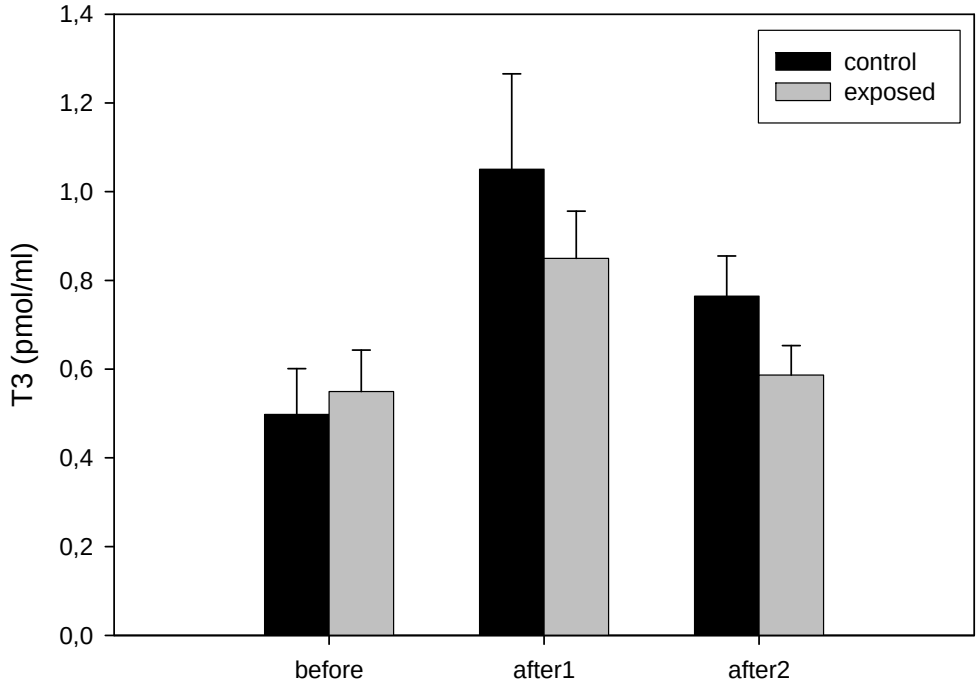

Figure 2: Triiodothyronin (T3) concentrations (pmol/ml) in control and exposed group before implantation, 10-14 days after implantations (after1) and ~2 months after implantation (after2).

Thyroid hormones are essential for the development and continued function of many organs and tissues, including the central nervous system, as well as being necessary for proper growth and metabolism in vertebrates. Effects of PBDEs on thyroid function have previously been reported in rodents[16]. Exposure of American kestrels before and after hatching to different PBDE congeners decreased T4 levels in the offspring[4]. PBDEs may interfere with thyroid function through different mechanisms of action. PBDEs have a high degree of structural resemblance to thyroid hormones and may therefore interfere with binding of thyroid hormones to receptors or transport proteins. Future studies with larger sample sizes should further investigate the effect of PBDEs on T3 in European starlings.

Biochemical parameters

For albumin, a repeated measures ANOVA revealed a significant effect of treatment, a significant effect of time and there was a trend for an interaction between treatment and time (Repeated measures ANOVA: treatment: $F_{1,14} = 6.00$, $p = 0.03$; time: $F_{2,30} = 6.63$, $p = 0.004$; treatment × time: $F_{2,30} = 2.90$, $p = 0.07$). Before implantation, there was no significant difference in concentrations between the control and exposed group (Tukey HSD: $p = 0.99$). About two weeks after implantation, albumin concentrations of the exposed group were significantly lower compared to the control group (Tukey HSD: $p = 0.03$; Figure 3). About two weeks after implantation, concentrations were significantly lower compared to the concentrations before implantation in both the control and exposed group (Tukey HSF: $p < 0.03$; Figure 3). Post-hoc tests revealed no other significant differences.

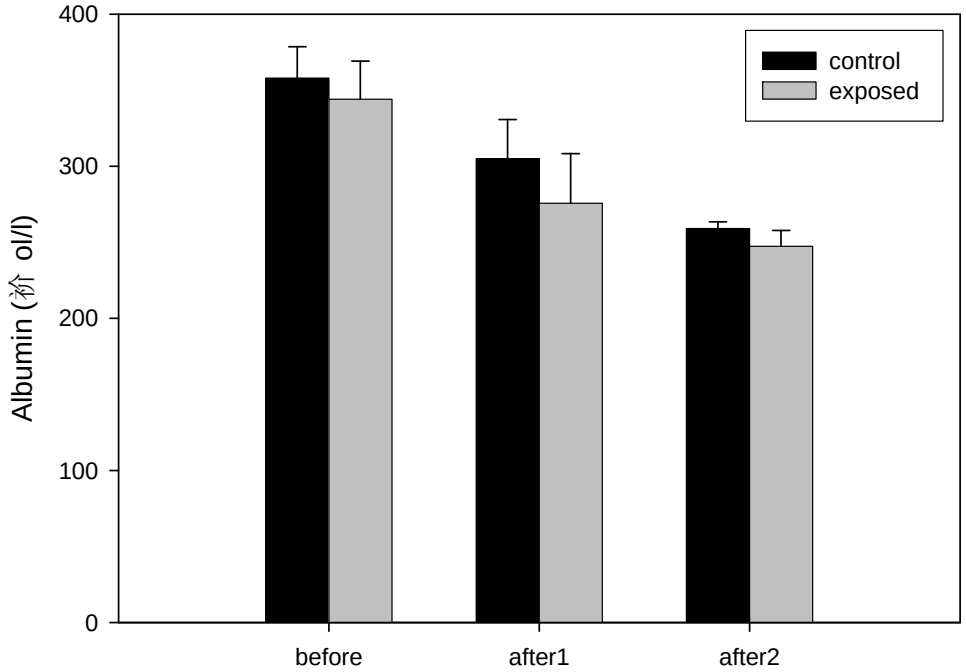

Figure 3: Albumin concentrations (µmol/l) in control and exposed group before implantation, 10-14 days after implantations (after1) and ~2 months after implantation (after2).

Albumin serves as an important thyroid hormone carrier protein in birds. It is synthesized in the liver and is a highly sensitive and specific indicator of hepatocyte function[17,18] and chronic damage. Decreases occur with decreased synthesis (chronic liver disease, dietary protein deficiency or chronic inflammation), increased loss (renal disease, intestinal parasitism or gastrointestinal disease), or sequestration (decreased oncotic pressure or increased hydrostatic pressure). Decreases can also occur with blood loss, severe inanition and chronic infection. Negative correlations between albumin and PCB concentrations have previously been found in herring gulls (*Larus argentatus*)[19], pigeons[20] and great blue herons (*Ardea herodius*)[21].

For the triglycerides, there was a significant effect of treatment and time (Repeated measures ANOVA: treatment: $F_{1,19} = 5.72$, $p = 0.03$; time: $F_{2,40} = 6.53$, $p = 0.004$), but no interaction was found between treatment and time (Repeated measures ANOVA: $F2,40 = 0.84$, $p = 0.44$). Triglyceride concentrations were significantly lower in the exposed group compared to the control group (Tukey HSD: $p = 0.01$; Figure 4). About two weeks after implantation, triglyceride concentrations of the exposed group were significantly lower than the control group (Unpaired t-test: $t = 1.35$, $p = 0.0001$; Figure 4). Before implantation and about two months after implantation no significant differences were found between the groups (Unpaired t-tests: $t > 0.51$, $p > 0.09$).

About two months after implantation concentrations in both groups were significantly lower compared to the concentrations before implantation and 10-14 days after implantation (Tukey HSD: $p < 0.01$; Figure 4).

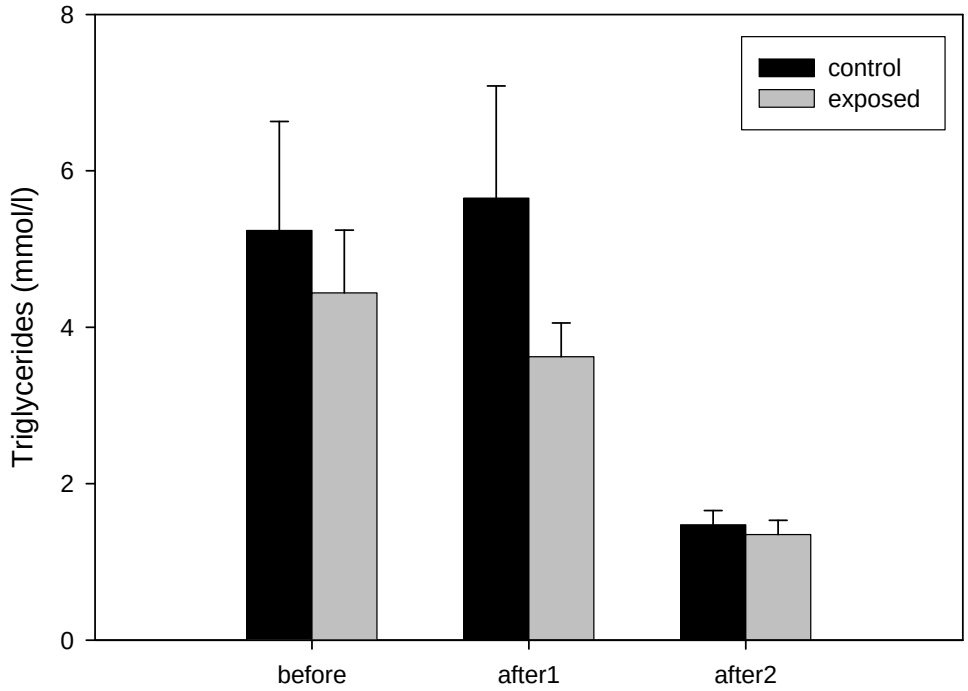

Figure 4: Triglyceride concentrations (mmol/l) in control and exposed group before implantation, 10-14 days after implantations (after1) and ~2 months after implantation (after2).

Triglycerides are a measure of lipid metabolism, transport and reserves[22,23]. Triglyceride concentrations were inversely correlated with PCBs in herring gulls from the Great Lakes[19]. To our knowledge no effects on albumin and triglyceride concentrations have been reported for PBDEs until now.

There were no significant differences found between the control and exposed group for the other biochemical parameters (total protein, uric acid, cholesterol, AST, total antioxidative capacity, HDL and creatine kinase; Repeated measures ANOVAs: treatment: $p > 0.05$; treatment × time: $p > 0.05$).

Our results suggest that albumin and triglyceride may be useful as non-specific biomarkers for contamination with PBDEs. Further research is also needed to investigate the mechanisms of action and to assess the impact of these effects on an individuals' health.

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