ACCUMULATION, TISSUE-SPECIFIC DISTRIBUTION AND BIOTRANSFORMATION OF BDE 209 IN EUROPEAN STARLINGS (*STURNUS VULGARIS*) AFTER EXPOSURE WITH SILASTIC IMPLANTS

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Introduction

Polybrominated diphenyl ethers (PBDEs) belong to the large family of brominated flame retardants, which are used as additives in plastics and textiles. Because of their persistent, bioaccumulative and toxic characteristics, the pentaBDE and octaBDE commercial mixtures have been withdrawn from the European market in 2004[1]. These products are now also facing bans in several states in the US, and will be removed from the North American market by 2008[2]. At present, the decaBDE commercial mixture is the only PBDE product which is still allowed for use. DecaBDE comprises approximately 80% of the world market demand for PBDEs, which in 2001 was reported at 56 100 metric tons[3]. The congener 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether (BDE 209) is the primary component of the decaBDE commercial mixture.

Because of the high molecular weight and hydrophobicity of BDE 209, it is assumed that BDE 209 is not easily absorbed by the gut and that it consequently has a low oral bioavailability[4]. However, BDE 209 has been identified in several animals, including birds[5]. Humans are also exposed, as evidenced by the detection of BDE 209 in the blood of occupationally exposed and unexposed individuals[6,7]. Recent dietary exposure studies with fish[8,9] and rats[10] have shown that BDE 209 is absorbed from the diet and that it can also be debrominated by metabolic routes. Several studies have shown an inverse relationship between the potential toxicity and the number of bromine atoms among the BDE congeners[11]. Therefore, the major concern with respect to the debromination of BDE 209 is that these lower brominated congeners can be more toxic and bioaccumulative than the mother compound.

The aim of the present study was to investigate the accumulation and tissue-specific distribution of BDE 209 in a terrestrial bird species, the European starling (*Sturnus vulgaris*). Attention was hereby also given to the biotransformation of BDE 209, with respect to its possible debromination to lower brominated congeners.

Materials and Methods

Exposure

Seven adult male starlings were exposed to BDE 209 through implanted silastic tubes (Degania silicone). BDE 209 was dissolved in isooctane and mixed in peanut oil (Sigma-Aldrich). The isooctane was removed by gently heating (40°C) the mixture until a constant weight was obtained. There were no detectable levels of other PBDE congeners in the oil solution. The implants were inserted under the skin by a small incision to lie alongside the ribs. The exposure group (n = 4) received an implantation dose of 46.8 ± 2.2 µg BDE 209 and the control group (n = 3) received an implant filled with only peanut oil. Blood concentrations of BDE 209 were monitored by taking every 3 to 7 days blood samples (~300 µl). After an exposure period of 76 days (16 sampling dates), the birds were sacrificed and the pectoral muscle and the liver were excised. Samples were stored at –20°C until further treatment.

Extraction and clean-up

The method for whole blood analysis was adapted from the method described by Covaci and Voorspoels[12] for the determination of PBDEs in serum. Approximately 300 µl of blood was spiked with internal standard (^{13}C-BDE 209), mixed with formic acid and extracted using solid-phase extraction cartridges (Oasis® HLB, Waters Corp.). Clean-up was done by column chromatography on silica impregnated with concentrated sulfuric acid (48

%, *w/w*). The cleaned extract was concentrated to 100 µl under a gentle nitrogen stream and transferred to an injection vial.

Because of the low contamination levels, we pooled the tissue samples of all individuals of the control group. In the exposure group, we pooled the tissues per 2 individuals. The method used for analysis of biological tissues has previously been described[13] and is briefly presented below. Three to 6 g of homogenized sample was chemically dried using anhydrous Na_2SO_4, transferred into an extraction thimble and spiked with internal standards (BDE 77, BDE 128 and ^{13}C-BDE 209). Extraction was carried out with 100 ml hexane/acetone (3:1, *v/v*) in an automated Soxhlet extractor (Büchi) in hot extraction mode for 2 h. The lipid content was determined gravimetrically on an aliquot of the extract, while the rest of the extract was cleaned on a column filled with 15 ml hexane and 10 ml dichloromethane. The eluate was concentrated to 100 µl under a gentle nitrogen stream and transferred to an injection vial.

Chemical analysis
An Agilent 6890 gas chromatograph was connected via direct interface with a 5973 mass spectrometer (Agilent Technologies). A 5 m x 0.18 mm x 0.18 µm DB-1 (J&W Scientific) capillary column was used with helium as carrier gas at a constant flow of 0.8 ml/min. The mass spectrometer was operated in electron-capture negative ionization mode. During the whole chromatographic run, ions *m/z* = 484.7/486.7 and 494.7/496.7 were monitored for BDE 209 and ^{13}C-BDE 209, while ions *m/z* = 79 and 81 were monitored for other PBDEs.

Quality Control
Procedural blanks, which consisted of water instead of the blood sample, were included with each sample batch and values obtained for the blanks were subtracted from the values found in the samples. External quality control is assessed through regular participation with good results (deviation from target values <20%) to the Arctic Monitoring and Assessment Programme (AMAP) and to the QUASIMEME proficiency exercises for PBDEs in environmental samples.

Results and Discussion
Whole blood concentrations of BDE 209

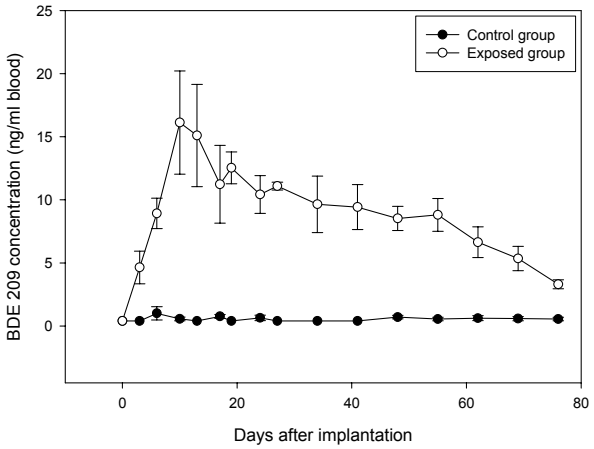

Figure 1: Mean blood concentrations of BDE 209 with standard errors (ng/ml blood) of the control group (n = 3) and the exposed group (n = 4) after implantation with BDE 209.

Before implantation, BDE 209 concentrations in the blood were below the limit of quantification (LOQ; 0.8 ng/ml blood) in both the control group and the exposed group. Mean concentrations in the control group varied between < 0.8 ng/ml blood and 1.0 ± 0.5 ng/ml blood throughout the exposure period (Fig. 1). During the first week of exposure, BDE 209 accumulated in the blood of the starlings from < 0.8 ng/ml to a mean peak concentration of 16.1 ± 4.1 ng/ml blood on day 10 (Fig. 1). After this peak, there was a decline in BDE 209

concentrations to 3.3 ± 0.4 ng/ml blood (Fig. 1), which suggest biotransformation of BDE 209. However, during the exposure period, no lower brominated BDE congeners were detected in the blood. It may be that lower brominated congeners were present, but, due to the low sample volume available, the concentrations were below the LOQ.

BDE 209 concentrations in muscle and liver

Tissue concentrations were below the LOQ in the control group (Muscle: < 5.6 ng/g lipid weight, Liver: < 2.9 ng/g lipid weight). In the exposed group, the muscle concentrations were about two times higher than the liver concentrations (Muscle: 445 ng/g lipid weight, Liver: 237 ng/g lipid weight). The higher concentrations in the muscle compared to the liver are probably due to a higher biotransformation rate in liver than in muscle. In addition to BDE 209, Fig. 2 shows that other PBDE congeners were present in the tissues. The profiles of muscle and liver for the other PBDE cogneners were similar (Fig. 2). Highest differences between the control group and the exposed group were observed for octa- and nona-BDEs, suggesting bioformation from BDE 209 (Fig 2). Penta-, hexa-, and hepta-BDEs differed less between the control group and the exposed group (Fig. 2). There were no differences observed between the control group and the exposed group for BDE 47, 100, 154, 203 and 205 (Fig. 2). A possible explanation for the differences in the formation of the octa-BDEs is the position of the bromine atoms.

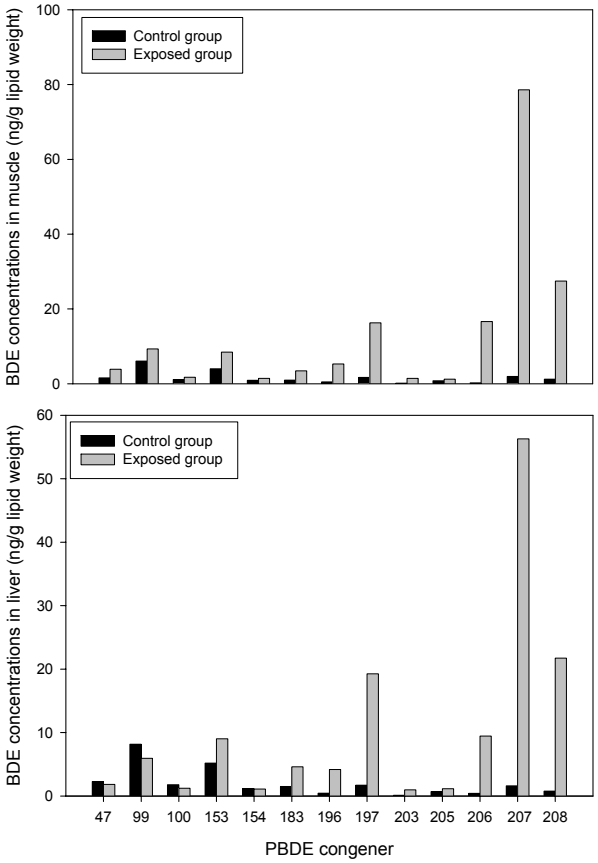

Figure 2: Profiles of PBDE congeners in muscle and liver after implantation with BDE 209.

Since there were no other PBDE congeners detected in the BDE 209 oil solution used in the implants, the selective accumulation of minor components from the BDE solution is very implausible. Our results suggest that BDE 209 is degraded to lower brominated congeners in tissues of European starlings. The bioformation of lower brominated congeners from decaBDE has previously been reported in fish[8,9] and rats[10]. Similar as in fish[8,9], our results suggested that BDE 209 can be debrominated down to hexa-BDEs. For the nona-BDEs, we observed the following abundance BDE 207 > BDE208 > BDE 206. A similar pattern was found resulting from thermal

decomposition of BDE 209[14], while other patterns were observed for photolytic degradation[15] or biotransformation in fish[10]. To our knowledge, these results are the first indications for the biotransformation of BDE 209 in birds.

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