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Levels of PBDEs in plasma of juvenile starlings (*Sturnus vulgaris*) from British Columbia, Canada and assessment of PBDE metabolism by avian liver microsomes

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HIGHLIGHTS

- The Delta landfill is clearly a source of PBDEs for starling chicks.
- BDE-47 and BDE-99 are the major PBDEs in starling chick plasma.
- *In vitro* and *in vivo* metabolism of BDE-47 and BDE-99 in starling is very slow.
- Differences in BDE-47 and BDE-99 *in vitro* metabolism by human, rat and starling exist.

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ABSTRACT

In this study, the levels of polybrominated diphenyl ethers (PBDEs), HO-PBDEs, and bromophenols were monitored in starling chick plasma samples collected in Delta (British Columbia, Canada) close to the Vancouver municipal landfill and in Glen Valley, a rural area in British Columbia. The *in vitro* formation of hydroxylated metabolites of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) was also investigated using starling chick liver microsomes. Total PBDE plasma levels were approximately 60 times higher in starling chicks from Delta than from Glen Valley, suggesting that the Delta site is a major source of PBDEs for the local population of starlings and that PBDEs previously measured in starling eggs are bioavailable to chicks. In both locations, BDE-47 and BDE-99 were the two major congeners present at similar concentrations, suggesting contamination with the Penta-BDE mixture. Among the several possible hydroxylated metabolites of PBDEs monitored in starling plasma, only 2,4,5-tribromophenol was detected and its levels did not exceed 18 ± 7 pg/mL. Also, several hydroxylated metabolites of BDE-47 and BDE-99 were formed by starling chick liver microsomes, but in low amounts. Therefore, our data consistently suggest that oxidative metabolism of PBDEs in starling chicks proceeds at low rate *in vivo* and *in vitro*. In conclusion, the landfill located in Delta is a relevant source of bioavailable PBDEs for the local starling population. Because of the limited ability of starling chicks to metabolize PBDEs, these compounds are likely to bioaccumulate in starlings over time. The possible toxicological implications of PBDEs bioaccumulation in starlings are currently unknown and require further research.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants worldwide for over 40 years (Vonderheide et al., 2008). PBDEs have been added to many polymers present in everyday consumer products, such as electronics, furniture and paints (Alaee et al., 2003). Because PBDEs have been shown to leak from the products they are applied to, they have become ubiquitous environmental pollutants (Hites, 2004). 2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47) and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) were the two major congeners

Abbreviations: BDE-47, 2,2′,4,4′-Tetrabromodiphenyl ether; BDE-99, 2,2′,4,4′,5-Pentabromodiphenyl ether; CYP, Cytochrome P450; CMP, Environment Canada Chemical Management Plan; GC-ECNI/MS, Gas chromatography–electron capture negative ionization mass spectrometry; HO-PBDEs, Hydroxylated PBDEs; LOQ, Limit of quantification; NADPH, Nicotinamide adenine dinucleotide phosphate; PBDEs, Polybrominated diphenyl ethers; SPE, Solid-phase extraction.

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in one of the most commonly used PBDE commercial mixtures known as Penta-BDE (LaGuardia et al., 2006).

PBDEs have been detected in sediment, crab (*Cancer magister*), sockeye salmon (*Oncorhynchus nerka*), king salmon (*Oncorhynchus tshawytscha*) and seal (*Pusa hispida*) samples from British Columbia (Ikonomou et al., 2002, 2006; Kelly et al., 2011). In 2008, the Environment Canada Chemical Management Plan (CMP; http://www.chemicalsubstanceschimiques.gc.ca/plan/index-eng.php) established a terrestrial monitoring program using eggs of the European starling (*Sturnus vulgaris*) in a cross-Canada program to track spatial and temporal trends in emerging contaminants and determine their sources. Over the last four years, elevated concentrations of PBDEs (up to 4.4 μg/g lipid weight) were found in starling eggs collected in the Vancouver municipal landfill area (Delta, British Columbia; Chen et al., 2013; Eens et al., 2013), suggesting that the Vancouver municipal landfill is a point source of PBDEs for the local population of starlings. However, no information about the bioavailability of the PBDEs present in starling eggs (i.e. the ability of PBDEs present in eggs to reach the blood circulation) and their metabolism is presently available.

Birds have been used as sentinel species for biomonitoring of environmental contaminants in the trophic chain since they are exposed to organohalogenated pollutants like PBDEs mainly via dietary intake. In particular, for these purposes starlings are an ideal bird species in terrestrial environments because they have small home ranges and forage primarily on soil invertebrates in the immediate area surrounding their nests (Dauwe et al., 2006; Eens et al., 2013; Moore, 1966). Therefore, starling is an ideal bird species to monitor site-specific accumulation and bioavailability of soil contaminants, such as PBDEs, present also in invertebrates.

Hydroxylated metabolites of PBDEs are potential endocrine disrupting chemicals which may be able to alter thyroid hormone homeostasis. Recently, in vitro studies have shown that hydroxylated PBDEs (HO-PBDEs) can be more potent that PBDEs in altering the mechanisms regulating the thyroid hormone levels in mammals (Cao et al., 2010; Hamers et al., 2008; Li et al., 2010; Marchesini et al., 2008; Ren and Guo, 2012). HO-PBDEs have been detected in plasma samples of peregrine falcon (*Falco peregrinus*), bald eagles (*Haliaeetus leucocephalus*) and glaucous gull (*Larus hyperboreus*) suggesting that PBDEs are oxidatively metabolized in these bird species (Fernie and Letcher, 2010; McKinney et al., 2006; Verreault et al., 2005). In contrast, no information about in vivo and in vitro PBDEs metabolism in starlings is presently available. Identifying and measuring the levels of the hydroxylated metabolites of PBDEs present in starling blood and formed in vitro by starling liver microsomes would contribute significantly to the toxicological risk assessment of PBDEs in starlings.

The objectives of the present study were (i) to investigate if the PBDEs present in starling eggs are bioavailable for the developing chicks measuring PBDE levels in starlings plasma, (ii) to determine if the Delta site is a major source of bioavailable PBDEs for local birds comparing the PBDE levels in plasma of starling chicks collected from Delta landfill with those collected in a rural area, 40 km east of Delta, in Glen Valley, British Columbia and (iii) to determine the role that metabolism might have in explaining the plasma concentration of PBDEs in juvenile starlings investigating the in vivo and in vitro metabolism of PBDEs in starlings.

2. Materials and methods

2.1. Chemicals and reagents

Standards for 2,2′,4-tribromodiphenyl ether (BDE-28), 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153) and 2,2′,4,4′,5,6-hexabromodiphenyl ether (BDE-154) were purchased from Wellington Laboratories (Guelph, Ontario — Canada). Standards for 4′-hydroxy-2,2′,4-tribromodiphenyl ether (4′-OH-BDE-17), 2′-hydroxy-2,4,4′-tribromodiphenyl ether (2′-OH-BDE-28), 4-hydroxy-2,2′,3,4′-tetrabromodiphenyl ether (4-OH-BDE-42), 3-OH-2,2′,4,4′-tetrabromodiphenyl ether (3-OH-BDE-47), 5-OH-2,2′,4,4′-tetrabromodiphenyl ether (5-OH-BDE-47), 6-OH-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE-47), 4′-OH-2,2′,4,5′-tetrabromodiphenyl ether (4′-OH-BDE-49), 4-hydroxy-2,2′,3,4′,5-pentabromodiphenyl ether (4-OH-BDE-90), 5′-hydroxy-2,2′,4,4′,5-pentabromodiphenyl ether (5′-OH-BDE-99), 6′-hydroxy-2,2′,4,4′,5-pentabromodiphenyl ether (6′-OH-BDE-99), 4′-hydroxy-2,2′,4,5,5′-pentabromodiphenyl ether (4′-OH-BDE-101), and 2,4,5-tribromophenol (2,4,5-TBP) (10 or 50 μg/mL in acetonitrile, 97.7% grade purity or higher for all the standards mentioned) were obtained from AccuStandard (New Haven, CT). Standards for 2,4-dibromophenol (2,4-DBP) and NADPH were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). The standard for 2-hydroxy-2 ,3,4,4 ,5-pentabromodiphenyl ether (2-OH-BDE-123; neat) was a generous gift from Dr. R.J. Letcher (Environment Canada, Ottawa, Canada). Solid-phase extraction (SPE) cartridges were obtained from Waters (Oasis HLB, 6 mL/500 mg) and Varian (Silica, 3 mL/500 mg). Hydrochloric acid, sodium hydroxide, and organic solvents (HPLC grade or better) were purchased from Fisher Scientific (Ottawa, Ontario, Canada). Ultra-pure water was prepared using a Millipore Milli-Q system (Billerica, MA). Pooled human liver microsomes (mixed gender, n = 50) were purchased from Xenotech (Lenexa, Kansas — U.S.). Rat liver microsomes were prepared as previously described from rats treated with corn oil (Erratico et al., 2010).

2.2. Collection of starling liver and plasma samples

Sample collections were carried out between April and July 2012 at the Vancouver Municipal Landfill in Delta (49° 5′ 58.1964″ N, 123° 0′ 10.5480′ W) and at the Glen Valley Farm in Fort Langley (49° 9′ 13.62224″ N, 122°28′ 4.4544″ W) under a Simon Fraser University Animal Care permit (829B-96). Blood and liver samples were collected from 17-day-post-hatch starling chicks in Delta (n = 5 and n = 2, respectively) and in Fort Langley (n = 9 and n = 1, respectively). Birds were euthanized using a rompun:ketamine 50:50 mixture solution. The blood was collected using a Fisherbrand Microhematocrit capillary tube internally coated with an ammonium heparin, transferred to an Eppendorf tube and placed immediately on ice. Blood samples were collected at the end of the day so that they could be processed within 90 min at the laboratory. Euthanized chicks were also placed on ice and transferred to the laboratory at The Simon Fraser University, where the livers were removed and stored in a previously prepared hexane:acetone rinsed glass vial. Plasma was obtained centrifuging blood samples at 13,000 rpm for 5 min. Pooled plasma (Delta n = 5, Fort Langley n = 9) and the liver (Delta n = 2, Fort Langley n = 1) samples were stored at −80 °C.

2.3. Preparation of starling liver microsomes and determination of total protein content

Liver microsomes were prepared from pooled starling livers (Delta: n = 2, Glenn Valley: n = 1). Two pools were prepared, one for each sampling location. Liver microsomes were prepared as previously described (Thomas et al., 1983), suspended in 0.25 M sucrose solution and stored at −80 °C. Protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

2.4. Determination of PBDEs and HO-PBDEs in starling plasma

The plasma samples were analyzed using a previously described method (Weijs et al., 2009). Briefly, analytes in 1 mL triplicate aliquots