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Hepatic microsomal metabolism of BDE-47 and BDE-99 by lesser snow geese and Japanese quail

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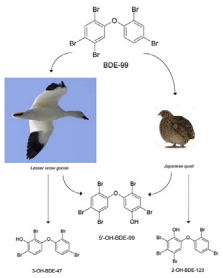
HIGHLIGHTS

- Hepatic metabolism of BDE-47 and BDE-99 by lesser snow geese and Japanese quail.
- Multiple monohydroxy- and dihydroxy-metabolites were detected by UHPLC/MS/MS.
- Species- and sex-specific differences in metabolite formation rates.
- Comparisons of metabolite profiles with other avian species and mammals.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, we investigated the oxidative biotransformation of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) by liver microsomes from wild lesser snow geese (*Chen caerulescens caerulescens*) and domesticated Japanese quail (*Coturnix japonica*). Formation of hydroxy-metabolites was analyzed using an ultra-high performance liquid chromatography-tandem mass spectrometry-based method. Incubation of BDE-47 with avian liver microsomes produced sixteen hydroxy-metabolites, eight of which were identified using authentic standards. The major metabolites formed by liver microsomes from individual lesser snow geese were 4-hydroxy-2,2′,3,4′-tetrabromodiphenyl ether (4-OH-BDE-42), 3-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (3-OH-BDE-47), and 4′-hydroxy-2,2′,4,5′-tetrabromodiphenyl ether (4′-OH-BDE-49). By comparison, 4-OH-BDE-42 and 4′-OH-BDE-49, but not 3-OH-BDE-47, were major metabolites of Japanese quail liver microsomes. Unidentified metabolites included monohydroxy- and dihydroxy-tetrabromodiphenyl ethers. Incubation of BDE-99 with avian liver microsomes produced seventeen hydroxy-metabolites, twelve of which were identified using authentic standards. The major metabolites formed by lesser snow goose liver microsomes were 2,4,5-tribromophenol, 3-OH-BDE-47, 4′-OH-BDE-49, 4-hydroxy-2,2′,3,4′,5-pentabromodiphenyl ether (4-OH-BDE-90), and 5′-hydroxy-2,2′,4,4′,5-pentabromodiphenyl ether (5′-OH-BDE-99). By comparison, the major metabolites produced by liver microsomes from Japanese quail included 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE-47) and 2-hydroxy-2′,3,4,4′,5-pentabromodiphenyl ether (2-OH-BDE-123), but not 3-OH-BDE-47. Unidentified metabolites consisted of monohydroxy-pentabromodiphenyl ethers, monohydroxy-tetrabromodiphenyl ethers and dihydroxy-tetrabromodiphenyl ethers. Another difference between the two species was that formation rates of BDE-47 and BDE-99 metabolites were

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greater with liver microsomes from male than female Japanese quail, but a sex difference was not observed with lesser snow geese.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are stable, highly lipophilic compounds that were used as additive flame retardants on household and industrial products. PBDEs were commercially formulated in mixtures called Penta-BDE, Octa-BDE and Deca-BDE, which differed in PBDE congener composition. Penta-BDE was used predominantly in North America for application to textiles, polyurethane foam and electronic components, and mainly consisted of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) and 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) (Alaee et al., 2003; Hale et al., 2003). Because PBDEs were not chemically bound to the products, they tend to leach from these products during their manufacture, use or disposal. As a consequence, PBDEs have become nearly ubiquitous environmental contaminants occurring in sediments, household dust, indoor air, humans and wildlife including birds (Law et al., 2003; Hites, 2004; Elliott et al., 2005; Verreault et al., 2005; McKinney et al., 2006; Hale et al., 2006; Park et al., 2009; Liu et al., 2010; Chen and Hale, 2010; Law et al., 2014).

Acute toxicity from environmental PBDE exposure in humans and wildlife has yet to be proven, but studies with laboratory animals have shown that developmental exposure to BDE-47 or BDE-99, which are among the dominant congeners found in abiotic and biotic samples (Law et al., 2003, 2014), can disrupt thyroid hormone activity (Zhou et al., 2001; Talsness et al., 2008; Kodavanti et al., 2010), disturb the reproductive system and behavior (Kuriyama et al., 2005; Lilienthal et al., 2006; Talsness et al., 2008; Cheng et al., 2009; Kodavanti et al., 2010), and alter neuromotor activity (Gee and Moser, 2008; Kodavanti et al., 2015). Decreased reproductive success appears to be a major adverse effect associated with environmental PBDE exposure in birds (Rattner et al., 2004). Treatment of captive adult American kestrels (*Falco sparverius*) with DE-71, a Penta-BDE mixture, was found to alter courtship behavior (Fernie et al., 2008) and *in ovo* treatment of American kestrels with DE-71 resulted in impaired pipping and hatching success (McKernan et al., 2009). Similarly, *in ovo* treatment with BDE-99 led to reduced clutch size in zebra finch (*Taeniopygia guttata*) (Winter et al., 2013). Additional adverse effects, including decreased plasma thyroxine, decreased vitamin A levels and increased hepatic oxidative stress, were observed in American kestrels that were treated *in ovo* and post-hatch with BDE-47, BDE-99, BDE-100 and BDE-153 (Fernie et al., 2005).

Some of these adverse effects could be linked to the *in vivo* biotransformation of PBDEs into hydroxylated metabolites. Hydroxy-PBDEs have been shown to be biologically active in *in vitro* studies (Meerts et al., 2001; Marchesini et al., 2008; Li et al., 2010; Dingemans et al., 2011; Butt et al., 2011). For example, hydroxy-PBDEs bind with higher affinity to the thyroid hormone transport protein, transthyretin, and to the estrogen receptor than several PBDE congeners (Mercado-Feliciano and Bigsby, 2008; Hamers et al., 2008; Cao et al., 2010; Ren and Guo, 2012). Hydroxy-PBDEs have been identified in blood samples from free-ranging birds (Verreault et al., 2005; McKinney et al., 2006; Liu et al., 2010; Dahlberg et al., 2016). A possible source of these hydroxy-PBDEs is dietary intake of naturally-occurring hydroxy-PBDEs, but it is more likely that they originated from the *in vivo* metabolism of PBDEs catalyzed by hepatic cytochrome P450 (CYP) enzymes. Hepatic microsomal biotransformation of PBDEs has been demonstrated in mammalian species including humans, rats and polar bears (Erratico et al., 2011; Erratico et al., 2012, 2013; Feo et al., 2013; Krieger et al., 2016). Moreover, these studies showed that oxidative biotransformation of BDE-47 and BDE-99 *via* aromatic hydroxylation, oxidative debromination, dealkylation and dihydroxylation reactions, which were catalyzed by hepatic CYP enzymes, produced a variety of hydroxylated metabolites (Erratico et al., 2011; Erratico et al., 2012, 2013; Feo et al., 2013). Considerably less information is available regarding hepatic PBDE biotransformation by avian species. Oxidative biotransformation of BDE-47 and BDE-99 by liver microsomes prepared from chickens (*Gallus gallus domesticus*) and European starlings (*Sturnus vulgaris*) was reported recently (Zheng et al., 2015; Erratico et al., 2015), but it is unclear whether species or sexually dimorphic differences in metabolite formation occur in birds.

The lesser snow goose (*Chen caerulescens caerulescens*) is a large, typically white, migratory goose that breeds in the arctic regions of North America. It is designated as a subspecies of snow goose (*Chen caerulescens*) and differentiated from the greater snow goose (*Chen caerulescens atlanticus*) on the basis of size and geography. For example, the breeding grounds of the lesser snow goose range from Hudson Bay to the northern coast of Siberia, while the greater snow goose nests in the northeastern regions of Canada and western regions of Greenland (Syroechkovsky et al., 1994). There is considerable overlap, however, in size and geographical distribution between lesser and greater snow geese and this has led some to question the distinction between the subspecies. The Japanese quail (*Coturnix japonica*) is primarily a ground-living domesticated quail that is bred in many countries for egg and meat production (Mills et al., 1997).

The goal of the present study was to qualitatively and quantitatively compare oxidative biotransformation of BDE-47 and BDE-99 by liver microsomes from free-ranging lesser snow geese and from domesticated Japanese quail. Formation of hydroxy-PBDE metabolites was analyzed using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC/MS/MS). A secondary goal was to determine whether there are differences in metabolite formation between male and female birds.

2. Materials and methods

2.1. Chemicals and reagents

BDE-47 (neat, 100% purity), BDE-99 (neat, ≥ 99.2% purity), 2,4,5-tribromophenol (2,4,5-TBP), 4′-hydroxy-2,2′,4,6-tetrachlorobiphenyl (4′-OH-CB-50; neat, 99.9% purity), 4-hydroxy-2,3′,4,5′,6-pentachlorobiphenyl (4-OH-CB-121; neat, 99.9% purity) and hydroxy-PBDEs, including 3′-hydroxy-2,4,4′-tribromodiphenyl ether (3′-OH-BDE-28), were purchased from AccuStandard (New Haven, CT, USA) as reported previously (Erratico et al., 2013; Krieger et al., 2016). 2-Hydroxy-2′,3,4,4′,5-pentabromodiphenyl ether (2-OH-BDE-123) was a generous gift from Dr. R.J. Letcher (Environment Canada, Ottawa, ON, Canada). (A list of the hydroxy-PBDEs, including full names and abbreviations, analyzed in the present study can be found in the Supplementary material section). NADPH, sucrose and 2,4-dibromophenol (2,4-DBP) were purchased from