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Assessment of concentrations and effects of organohalogen contaminants in a terrestrial passerine, the European starling

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HIGHLIGHTS

- We measured PBDEs in European starling eggs from an agricultural area.
- We measured OCs and PCBs in a subset of eggs (n = 6) to assess background levels.
- ΣDDT and ΣPBDEs were highly variable among individual eggs from different nests.
- All contaminant concentrations were below levels previously reported to cause effects.
- ΣPBDEs in eggs were not related to success or condition of corresponding nests.

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ABSTRACT

European starlings (*Sturnus vulgaris*) are a valuable model species for the assessment of concentrations and effects of environmental contaminants in terrestrial birds. Polybrominated diphenyl ethers (PBDEs) are found in birds throughout the world, but relatively little is known of their concentrations or effects in free-living terrestrial passerines. We used a nest box population of European starlings to 1) measure the variation in egg concentrations of persistent organohalogen contaminants at an agricultural site, and 2) assess whether individual variation in PBDE concentrations in eggs was related to reproductive parameters, as well as maternal or nestling characteristics including body condition, thyroid hormones, oxidative stress, and hematocrit. As PBDEs were the main contaminant class of interest, we only assessed a subset of eggs for other organohalogen contaminants to establish background concentrations. Exposure to organohalogen contaminants was extremely variable over this relatively small study area. Geometric mean wet weight concentrations (range in brackets) of the major contaminants were 36.5 (12–174) ng/g ΣDDT (n = 6 eggs) and 10.9 (2–307) ng/g ΣPBDEs (n = 14). ΣPCBs at 3.58 (1.5–6.4) ng/g (n = 6) were lower and less variable. There were low levels of other organochlorine (OC) pesticides such as dieldrin (2.02 ng/g), chlordanes (1.11 ng/g) and chlorobenzenes (0.23 ng/g). The only form of DDT detected was *p,p′*-DDE. The congener profiles of PBDEs and PCBs reflect those of industrial mixtures (i.e. DE-71, Aroclors 1254, 1260 and 1262). For all of the contaminant classes, concentrations detected in eggs at our study site were below levels previously reported to cause effects. Due to small sample sizes, we did not assess the relationship between ΣPCBs or ΣOCs and adult or chick condition. We observed no correlative relationships between individual variation in PBDE concentrations in starling eggs and reproductive success, maternal condition, or nestling condition in the corresponding nests.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been widely used as additive flame retardants in plastics, textiles, foams, and electronic circuitry and are persistent and bioaccumulative, which contribute to their ubiquitous distribution in environmental, wildlife, and human samples (de Wit, 2002; Law et al., 2003). PBDEs have been found in avian tissue and egg samples throughout the world (Chen and Hale, 2010) but their emergence as environmental contaminants is relatively recent, as monitoring studies indicate that the increases in concentrations in the North

Abbreviations: BC, British Columbia; BCI, body condition index; CBz, chlorinated benzene; CHL, chlordanes; ELISA, enzyme-linked immunosorbent assay; FT3, free triiodothyronine; FT4, free thyroxine; HCH, hexachlorocyclohexane; OC, organochlorine; OCS, octachlorostyrene; OSI, oxidative status index; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyl ethers; *p,p′*-DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene; *p,p′*-DDT, dichlorodiphenyltrichloroethane; T3, triiodothyronine; T4, thyroxine; TAC, total antioxidant capacity; TCPM, tris(4-chlorophenyl)methanol; TOS, total oxidant status.

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American environment occurred primarily over the past 30 years (Chen and Hale, 2010; de Wit, 2002; Law et al., 2003). In Canada, regulations prohibit the manufacture of all PBDEs and restrict the use of penta-BDE and octa-BDE mixtures (Canada Gazette, 2008). However, PBDEs persist in the environment and will continue to leach from existing products that are in use or have been disposed of in landfills, and deca-BDE is still in use in Canada. Legacy persistent organic pollutants that have been heavily restricted in North America since the 1970s, such as polychlorinated biphenyl ethers (PCBs), dichlorodiphenyltrichloroethane (*p*,*p*′-DDT) and its metabolite 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*p*,*p*′-DDE), and other organochlorine (OC) pesticides, are also commonly detected in the environment. The presence of PBDEs, PCBs and OCs in the environment is a concern, as they are known to cause a wide range of toxicological effects in birds (Blus, 2011; Chen and Hale, 2010; Elliott and Bishop, 2011; Harris and Elliott, 2011). Penta- and octa-BDEs, PCBs, DDT and other OCs are all further regulated internationally under the Stockholm Convention on Persistent Organic Pollutants (POPs) (UNEP, 2011).

In the North American environment, there are several long-term datasets that have assessed the temporal and spatial variation of organohalogens, and specifically PBDEs, in aquatic birds and top predators (e.g. Braune et al., 2007; Elliott et al., 2005; Gauthier et al., 2008); however, much less is known about organohalogen (and particularly PBDE) concentrations in terrestrial passerines in North America. Our understanding of the toxic effects of PBDEs in birds is also limited relative to our knowledge of DDT and PCB toxicity. Studies that have assessed the effects of PBDE exposure in birds have primarily been in captive individuals (e.g. Eng et al., 2013; Fernie et al., 2005; Letcher et al., 2013; McKernan et al., 2009; Van den Steen et al., 2009a) or in top predators (e.g. Cesh et al., 2010; Henny et al., 2009; Verreault et al., 2007), and effects in free-living terrestrial passerines have received little attention. The European starling (*Sturnus vulgaris*) is a useful model passerine species for both monitoring local contamination and assessing consequent effects in terrestrial free-living birds. Starlings readily use nest boxes, which makes monitoring and sample collection for the purpose of linking contaminant exposure with biological responses relatively easy. Starlings have successfully been used as biological indicators of PCB effects (Arenal et al., 2004). Starlings have a widespread distribution across several habitats (urban, suburban, rural, agricultural) and throughout many regions of the world (Europe, North America, parts of Asia, Africa, and Australasia), which allows for within-species comparisons of contaminant levels across many different spatial scales.

Spatial variation in contaminants is often assessed at a broad scale, such as differences between regions or sites. However, there can be significant inter-individual variation even at a small scale, such as within a study site. European starling eggs have been used to study variation in terrestrial organohalogen contaminants at an intercontinental scale (Eens et al., 2013), and at a landscape scale (Chen et al., 2013). To assess inter-individual variation in contaminants at a smaller scale, eggs can be analyzed individually rather than by pooling samples. Previous studies of organohalogens in birds have shown that a single egg can be used as an indicator of nest contaminants (Custer et al., 1990; Van den Steen et al., 2006). By only collecting a single egg per nest, the growth, physiology and survival of remaining offspring in the nest can be monitored, and the productivity and condition of the individual nest can be related back to the contaminant levels in the egg.

The objectives of our study were 1) to measure the concentrations of PBDEs, PCBs and OCs in European starling eggs from different nests to assess the individual variation in background contaminant levels within a rural agricultural site, and 2) to relate individual variation in PBDE exposure to reproductive success and to measures of condition in the mothers and offspring, including body condition, thyroid hormones, oxidative stress, and hematocrit. Given the number of individual PCB and OC analytes, their measurement involves a considerable amount of analysis. As PBDEs were the contaminant class of interest, only a subset of eggs was initially analyzed for PCBs and OCs to establish background concentrations for our field site. If concentrations approached the thresholds for effects that are well established for PCBs and OCs, the remaining eggs would be analyzed. We found that the concentrations of PCBs and OCs in eggs at our site were ~2 to 3 orders of magnitude below the lowest concentrations known to cause effects in birds, so PCBs and OCs were not measured in the remaining egg samples.

2. Materials and methods

2.1. Study site

This research was carried out from May to June of 2009 at Wind's Reach Farm in Langley, British Columbia (BC), Canada (49° 9′ 16″N, 122° 28′ 22″W) under a Simon Fraser University Animal Care Committee permit (864B-08) in accordance with guidelines from the Canadian Committee on Animal Care. The site consists of approximately 60 wooden next boxes on farm buildings, fence posts, and trees, and is within the BC Agricultural Land Reserve.

2.2. Monitoring

All boxes were checked daily to determine clutch initiation and completion dates, laying sequence of the eggs, and hatching and fledgling success. We monitored 14 occupied nest boxes. The post-hatch nestling period is typically 21 days. Each egg was weighed (to the nearest 0.01 g) on the day it was laid. Nestlings were weighed and measured (tarsus length to the nearest 0.1 mm) at 0, 5, 10 and 15 days after hatching, and at 10 days of age nestlings were metal-banded. For each occupied nest box we recorded the number of parental nest visits for 30 min each day when nestlings were aged 6, 7 and 8 days, between 10:00 and 13:00. Provisioning rates were calculated per nestling per hour and based on the mean brood size of the nest for the 3-day observation period.

2.3. Blood sampling and egg collection

We caught female starlings ($n = 14$) while they roosted in their nest box at approximately 30 min before sunrise during late incubation (day 8 of 11-day incubation) to minimize possibility of nest abandonment. All birds were blood sampled (≤700 μl) from the brachial vein following puncture with a 26G needle within 3 min of capture. All females were measured (tarsus length and mass), and banded with metal and color bands. We returned birds to their nest boxes following sampling. Blood was collected into heparinized capillary tubes and stored on ice. Samples were centrifuged within 2 h to separate plasma from the red blood cells, and hematocrit was measured by packed cell volume. Plasma was stored frozen at 20 °C until analysis. Nestlings were blood sampled between 13:00 and 16:00 h on day 15 after hatching following the same methods, and returned to the nest box.

The second egg from the first clutch of each nest ($n = 14$) was collected to be used as an indicator of nest contaminant level. We collected the second egg, as sometimes the first egg laid is not viable. We consistently collected the same egg to control for any possible laying order effects when comparing across nests. In addition, it has been previously shown in birds that the variation in contaminant concentration within clutches is less than among clutches, and a single egg can be used as an indicator of nest contaminants (Custer et al., 1990; Van den Steen et al., 2006). Eggs were collected on the first day of incubation, using warmth as an indicator of incubation initiation. Whole eggs were removed from the shell and stored frozen (−80 °C) in chemically cleaned glass vials.