Science of the Total Environment 473–474 (2014) 589–596

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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

Margaret L. Eng [a,*], Tony D. Williams [a], Robert J. Letcher [b], John E. Elliott [c]

[a] Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada
[b] Wildlife and Landscape Science Directorate, Science and Technology Branch, Environment Canada, Carleton University, Ottawa, ON K1A 0H3, Canada
[c] Science and Technology Branch, Environment Canada, Pacific Wildlife Research Centre, Delta, BC V4K 3N2, Canada

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.

ARTICLE INFO

Article history:
Received 19 September 2013
Received in revised form 16 December 2013
Accepted 17 December 2013
Available online 4 January 2014

Keywords:
Polybrominated diphenyl ethers
Polychlorinated biphenyls
Organochlorine pesticides
Passerine eggs
Reproductive success
Physiology

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.

* Corresponding author. Tel.: +1 778 782 7398.
E-mail addresses: mea10@sfu.ca (M.L. Eng), tdwillia@sfu.ca (T.D. Williams), robert.letcher@ec.gc.ca (R.J. Letcher), john.elliott@ec.gc.ca (J.E. Elliott).

0048-9697/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.12.072

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 ($\leq$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.

2.4. Plasma analysis

Total and free thyroxine (T4 and FT4) and total and free triiodothyronine (T3 and FT3) levels in plasma samples were determined using enzyme-linked immunosorbent assay (ELISA) kits (Monobind 225-300, 1225-300, 125-300, and 1325-300, Lake Forest CA). We validated kits for parallelism and recovery using European starling plasma. In each plate we included hen plasma from a plasma pool to assess reproducibility and intra-assay precision, as well as quality control standards (Monobind ML-300) that we verified were in the expected range. The T4 assay had an average intra-assay coefficient of variation (CV) of 11.8% (n = 2 replicates) and the inter-assay CV was 8.2% (n = 3 assay plates). For FT4 the average intra-assay CV was 8.1% (n = 2 replicates) and the inter-assay CV was 11.8% (n = 2 plates). The average intra-assay CV for T3 was 1.8% (n = 2 replicates) and the inter-assay CV was 15.1% (n = 2 plates). For FT3 the average intra-assay CV was 12.7% (n = 2 replicates) and the inter-assay CV was 4.8% (n = 2 plates).

Total antioxidant capacity (TAC) was measured using a colorimetric method adapted from Erel (2004), where the colored 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation ($ABTS^{*+}$) is decolorized by antioxidants in the plasma according to their concentrations and antioxidant capacities. This reaction can be monitored spectrophotometrically, and the final absorbance is inversely related to the TAC of the sample. The reaction is calibrated using Trolox for the standard curve, and results are expressed as mmol Trolox equivalent/l. The total oxidant status (TOS) was measured using a colorimetric method adapted from Erel (2005), where oxidants in the plasma oxidize the ferrous ion–*o*-dianisidine complex to the ferric ion, which then reacts with xylenol orange to make a colored complex. The color intensity can be measured spectrophotometrically, and is proportional to the total amount of oxidant molecules in the plasma sample. The assay is calibrated using hydrogen peroxide (H_2O_2) for the standard curve, and the results are expressed in mmol H_2O_2 equivalent/l. The oxidative status index (OSI) for each individual was calculated as the ratio of TOS: TAS, with high ratios reflecting high oxidative stress. A hen plasma pool was used as an avian standard to compare intra- and inter-assay variation. The TAC assay had an average intra-assay coefficient of variation (CV) of 6.4% (n = 2 replicates) and the inter-assay CV was 11.9% (n = 4 assay plates). For TOS the average intra-assay CV was 8.7% (n = 2 replicates) and the inter-assay CV was 11.4% (n = 4 assay plates).

2.5. Chemical analysis

Egg samples were allowed to thaw at room temperature and homogenized using an Ultra Turrax High Performance disperser. All eggs (n = 14) were analyzed for 46 PBDE congeners, and a subset of eggs (n = 6) was analyzed for 74 PCB congeners and 21 different OC pesticide compounds. Details on the individual congeners/compounds determined are provided in footnotes (a) through (j) in Table 1. All PBDE, OC and PCB standards were purchased from Wellington Laboratories (Guelph, ON, Canada). Egg samples were accurately weighed, and neutral fractions were extracted and cleaned up using established methodologies (Gauthier et al., 2008). In brief, approximately 1 g of egg homogenate was subsampled and ground with 8–10 g of diatomaceous earth, and then extracted with 50% DCM/hexane using an accelerated solvent extraction system (Dionex ASE 200). The extraction columns were spiked with 20 μl of each internal standard (BDE-30, BDE-156, ^{13}C-labeled BDE-209, ^{13}C-PCB-28, ^{13}C-PCB-52, ^{13}C-PCB-118, ^{13}C-PCB-153, ^{13}C-PCB-180, ^{13}C-PCB-194, ^{13}C-β-hexachlorohexane, ^{13}C-octachlorostyrene, ^{13}C-*trans*-chlordane, ^{13}C-oxychlordane, ^{13}C-1,2,4,5-tetrachlorobenzene, ^{13}C-dieldrin, ^{13}C-BDE-209, ^{13}C-*trans*-nonachlor, ^{13}C-mirex, ^{13}C-α-hexachlorocyclohexane, ^{13}C-γ-hexachlorocyclohexane, ^{13}C-*cis*-chlordane, ^{13}C-*cis*-nonachlor, ^{13}C-*p,p′*-DDT, ^{13}C-*p,p′*-DDE, ^{13}C-*p,p′*-DDD, ^{13}C-heptachloroepoxide, ^{13}C-*p,p′*-TCPM, ^{13}C-hexachlorobenzene, and ^{13}C-pentachlorobenzene). The column extraction eluant was concentrated to 10 ml and a 10% portion was removed for gravimetric lipid determination. The remaining extracts were cleaned by gel permeation chromatography (GPC) and eluted from the GPC column with 50% DCM/hexane. The first fraction (140 ml) containing lipids and biogenic material was discarded, and the second fraction (200 ml) containing PBDEs, PCBs and OCs was concentrated to a volume of ~4 ml.

Table 1
Arithmetic mean (AM), geometric mean (GM), standard error (SE) and range of lipid percentage and sum (Σ) concentrations (ng/g wet weight) of organohalogen contaminants in European starling eggs.

	AM	GM	SE	Min.	Max.	N
% lipid	3.6	3.4	0.3	1.7	5.2	14
ΣDDT[a]	54.5	36.5	24.7	11.9	174.3	6
ΣPBDE[b]	46.9	10.9	25.6	2.0	307.4	14
ΣPCBs[c]	4.1	3.6	0.8	1.5	6.4	6
Dieldrin	2.0	2.0	0.1	1.5	2.3	6
ΣCHL[d]	1.2	1.1	0.2	0.7	1.6	6
ΣCBz[e]	0.2	0.2	0.03	0.2	0.3	6
ΣHCH[f]	nd[j]	nd	–	–	–	6
ΣMirex[g]	nd	nd	–	–	–	6
ΣTCPM[h]	nd	nd	–	–	–	6
ΣOCS[i]	nd	nd	–	–	–	6

[a] ΣDichloro-diphenyl-trichloroethane (ΣDDT): *p,p′*-DDE, *p,p′*-DDT, and *p,p′*-DDD.
[b] ΣPolybrominated diphenyl ether (ΣPBDE): BDE-1, 2, 3, 7, 10, 15, 17, 28, 47, 49, 54, 66, 71, 77, 85, 99, 100, 119, 138, 139, 140, 153, 154, 155, 170, 171, 179, 180, 181, 182, 183, 184, 188, 190, 191, 194, 195, 196, 197, 201, 202, 203, 205, 206, 207, 208, and 209.
[c] ΣPolychlroinated biphenyl (ΣPCB): CB-16/32, 17, 18, 22, 28, 31, 33/20, 42, 44, 47/48, 49, 52, 56/60, 64/41, 66, 70/76, 74, 85, 87, 92, 95, 97, 99, 101/90, 105, 110, 114, 118, 128, 130, 137, 138, 141, 146, 149, 151, 153, 156, 157, 158, 167, 170/190, 171, 172, 174, 176, 177, 178, 179, 180, 183, 187, 189, 194, 195, 196/203, 199, 200, 201, 202, 205, 206, 207, 208, and 209.
[d] ΣChlordanes (ΣCHL): heptachloroepoxide, oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, and *cis*-nonachlor.
[e] ΣChlorinated benzene (ΣCBz): 1,2,4,5-tetrachlorobenzene, 1,2,3,4,-tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene.
[f] Hexachlorocyclohexane (ΣHCH): α-hexachlorocyclohexane, β-hexachlorocyclohexane, and γ-hexachlorocyclohexane.
[g] ΣMirex: mirex and photomirex.
[h] ΣTris(4-chlorophenyl)methanol (ΣTCPM).
[i] ΣOctachlorostyrene (ΣOCS).
[j] Not detected. Limit of detection 0.1 ng/g ww.

All samples were cleaned up using a silica solid phase extraction (SPE) column (J.T. Baker, USA). The column was conditioned with successive washes of 10% (v/v) methanol (6 ml) in DCM and then 8 ml of 5% DCM in hexane. The sample was then loaded onto the cartridge and eluted with 8 ml of 5% DCM/hexane. The eluant was then concentrated and solvent exchanged with isooctane to a final volume of approximately 200 μl. The exact mass of each sample was recorded and the final volume determined by dividing the density of 2,2,4-trimethylpentane (0.69 g/ml).

PBDEs in the isolated chemical fractions were analyzed using gas chromatography–mass spectrometry working in electron capture negative ionization mode (GC/ECNI-MS). Analytes were separated and quantified on an Agilent 6890 series GC equipped with a 5973 quadrupole MS detector (Agilent Technologies, Palo Alto, CA). The analytical column was a 15 m × 0.25 mm × 0.10 μm DB-5HT fused-silica column (J & W Scientific, Brockville, ON, Canada). Helium and methane were used as the carrier and reagent gases, respectively. A sample volume of 1 μl was introduced to the injector operating in pulsed-splitless mode (injection pulse at 25.0 psi until 2 min; purge flow to split vent of 96.4 ml/min), with the injector held at 240 °C. The GC oven ramping temperature program was as follows: initial 100 °C for 2.0 min, 25 °C/min until 250 °C, 1.5 °C/min until 260 °C for 10.0 min, 25 °C/min until 325 °C and hold for a final 7.0 min. The GC to MS transfer line was held at 280 °C, ion source temperature was 250 °C, and the quadrupole temperature was 150 °C. Forty-eight BDE congeners were monitored using the bromine anions of *m/z* 79 and 81. Ions of *m/z* 487 and 489

were used for BDE-207, -208 and -209. Ions of *m/z* 485 and 487 were used to monitor BDE-197, -201 and -202. Ions of *m/z* 495 and 497 were used for the internal standard of $^{13}C_{12}$-labeled BDE-209.

OCs and PCBs in the isolated chemical fractions were analyzed using gas chromatography–mass spectrometry working in electron ionization mode (GC/EI-MS). Analytes were separated and quantified on an Agilent 6890 series GC equipped with a 5973 quadrupole MS detector The analytical column was a 30 m × 0.25 mm × 0.25 μm DB-5HT fused-silica column (J & W Scientific, Brockville, ON, Canada). Helium and methane were used as the carrier and reagent gases, respectively. For OCs, a sample volume of 1 μl was introduced to the injector operating in pulsed-splitless mode (injection pulse at 40.0 psi until 1 min; purge flow to split vent of 23.5 ml/min), with the injector held at 250 °C. The GC oven ramping temperature program was as follows: initial 100 °C for 3.0 min, 20 °C/min until 180 °C, and 5 °C/min until 300 °C. The GC to MS transfer line was held at 280 °C, ion source temperature was 230 °C, and the quadrupole temperature was 150 °C. For PCBs, a sample volume of 1 μl was introduced to the injector operating in splitless mode (purge flow to split vent of 53.7 ml/min to 1.5 min), with the injector held at 250 °C. The GC oven ramping temperature program was as follows: initial 100 °C for 3.0 min, 20 °C/min until 180 °C, and 2.5 °C/min until 300 °C. The GC to MS transfer line was held at 280 °C, ion source temperature was 230 °C, and the quadrupole temperature was 150 °C.

The analytes were identified on the basis of their retention times on the DB-5HT column, and verified by matching retention times with those of authentic standard mixtures. Mean internal standard recoveries were 78% ± 11% for BDE-30 and -156, 54 ± 22% for BDE-209, 94% ± 11% for OCs, and 97% ± 25% for PCBs. Analytes were quantified using an internal standard approach, thus all reported values were inherently recovery-corrected.

The limits of detection (LODs) for PBDE, OC and PCB analysis, based on a signal-to-noise ratio of 3, were 0.2, 0.1 and 0.06 ng/g wet weight (ww), respectively, with the exception of a LOD of 0.6 ng/g ww for dieldrin. Method blanks were included for each sample batch to assess background interference and possible contamination, and a blank subtraction was done for BDE-2, 7, 10, 15, 17, 28, 47, 49, 54, 66, 71, 77, 99, 100, 153, 154, 155, 183, 195, 196, 197, 203, 206 and 209, as well as for CB-66, 99, 101/90, 118, 138, 153, 170/190, 180 and 187, and a blank subtraction was done for hexachlorobenzene and *p,p′*-DDE. In-house standard reference material (double-crested cormorant [*Phalacrocorax auritus*] egg and Lake Michigan fish tissue) was also included in each sample batch to ensure consistency of data acquisition (within two SDs of in-house mean).

2.6. Statistical analysis

All statistical analyses were carried out using SAS 9.1.3 (SAS Institute, 2003). Data were tested for normality following Shapiro–Wilk tests, and by inspecting q–q plots. Variables that did not approximate the normal distribution (ΣPBDE, ΣDDT) were log-10 transformed prior to analysis to approximate a normal distribution. There was no correlation between egg contaminants and gravimetrically determined lipid content ($p \geq 0.084$), so the contaminant concentrations were not lipid normalized. Where appropriate, contaminants were analyzed as sums of closely related congeners and compounds based on their chemical structure. The contaminants quantified in this study were categorized as follows: ΣPBDE (n = 46 congeners), ΣPCB (n = 74 congeners), chlorinated benzene (ΣCBz, n = 4 congeners), chlordanes (ΣCHL, n = 6 compounds), DDT (ΣDDT, n = 3 compounds), hexachlorocyclohexane (ΣHCH, n = 3 isomers), mirex (n = 2 compounds), tris(4-chlorophenyl)methanol (TCPM), octachlorostyrene (OCS), and dieldrin. Compounds that were not detected in any of the samples were not included in further statistical analysis, and for the remaining compounds the samples with concentrations below the LOD were assigned a random value between zero and the compound-specific LOD to avoid missing values for statistical purposes. An index of body condition was estimated as the residuals from a linear regression of body mass on tarsus length (Schulte-Hostedde et al., 2005). The relationships between ΣPBDE concentrations and adult characteristics were assessed using generalized linear models (proc GLM). The relationships between ΣPBDE and chick characteristics were assessed using nest means, and brood size and provisioning rate of the nest were included as covariates in the GLMs. Due to small sample sizes, we did not assess the relationship between ΣPCBs or ΣOCs and adult or chick characteristics.

3. Results

The contaminant class with the highest geometric mean egg concentration was ΣDDT, followed by ΣPBDEs, then ΣPCBs, then other organochlorine pesticides (Table 1). All of the ΣDDT was made up of *p,p′*-DDE, with *p,p′*-DDT and *p,p′*-DDD not being detected. The major PBDE congeners in starling eggs, in descending order, were BDE-99, -47, and -100 which were found in all of the eggs, followed by BDE-153, -154, and -209 (Table 2). All other PBDE congeners were either not detected or contributed less than 1% of the ΣPBDEs. CB-153, -138, -187, -180, and -146 were the most abundant PCB congeners (22%, 19%, 15%, 8%, and 6% of the ΣPCBs, respectively). Of the chlordanes, heptachloroepoxide, oxychlordane, *trans*-nonachlor and *cis*-nonachlor were detected. Hexachlorobenzene was the only chlorobenzene detected. Many of the organochlorine pesticides (HCH, mirex, TCPM, OCS) were not detected in the starling eggs. *p,p′*-DDE was not significantly correlated with ΣPBDEs ($r = 0.57$, $p = 0.24$) or ΣPCBs ($r = 0.62$, $p = 0.19$). None of the other organochlorine pesticide classes were correlated with either ΣPBDEs ($p \geq 0.102$) or ΣPCBs ($p \geq 0.191$). There was a correlation between ΣPBDEs and ΣPCBs ($r = 0.876$, $p = 0.022$), although this is not significant after Bonferroni correction ($\alpha = 0.05/15 = 0.0033$).

Among the contaminant classes, ΣPBDEs had the broadest range of concentrations (Table 1), with most eggs (79%) having less than 10 ng/g ww, and three eggs (21%) having more than 50 ng/g ww. ΣPBDEs in the egg were not related to egg lipid ($r = 0.478$, $p = 0.084$) or egg mass ($r = -0.323$, $p = 0.261$). ΣPBDE concentrations in the eggs from European starlings were not related to any of the measured maternal characteristics (Fig. 1), including body condition ($F_{1,12} = 0.76$, $p = 0.399$), thyroid hormones ($p \geq 0.097$), oxidative stress ($F_{1,12} = 0.45$, $p = 0.516$), or hematocrit ($F_{1,12} = 1.23$, $p = 0.289$). ΣPBDEs in eggs were also not related to any of the measured reproductive parameters, including lay date, clutch size, clutch mass, average egg mass, or the number of fledglings per brood ($p \geq 0.513$). Similarly, PBDE concentrations in eggs were independent of characteristics of chicks from corresponding nests, including body condition at day 15 ($F_{1,10} = 0.86$, $p = 0.376$), thyroid hormones ($p \geq 0.122$), oxidative stress ($F_{1,10} = 0.05$, $p = 0.829$), or hematocrit ($F_{1,10} < 0.01$, $p = 0.981$).

4. Discussion

In the present study, we found detectable levels of PBDEs, *p,p′*-DDE and PCBs in every European starling egg that we measured in a rural agricultural site. The mean concentration of ΣDDTs was higher than that of ΣPBDEs and ΣPCBs, which is likely related to the agricultural landscape in which our field site is located. Concentrations of PCBs and PBDEs in passerines have been previously linked to urban and industrialized areas, while DDT and other OC pesticides have been linked to rural and agricultural areas (Eens et al., 2013; Sun et al., 2012; Van den Steen et al., 2008, 2009b). Very recently Chen et al. (2013) reported on PBDEs and other flame retardants (FRs) in fresh European starling eggs collected in 2009, 2010 and 2011 from nest boxes established within, adjacent to, and 10 and 40 km distant to five major urban centers across Canada. The data revealed orders of magnitude greater PBDE concentrations in eggs from starlings nesting in landfill sites relative to those from urban industrial and rural environments. In the

Table 2
Arithmetic mean (AM), geometric mean (GM), standard error (SE) and range of concentrations (ng/g wet weight) of PBDE congeners that made up greater than 1% of the sum (Σ) PBDEs in European starling eggs. Limit of detection (LOD) for PBDEs was 0.2 ng/g ww.

	AM	GM	SE	Min.	Max.	% of ΣPBDE (based on AM)	% of ΣPBDE (based on GM)	% n > LOD	Log K_{ow}[a]
ΣPBDE	46.9	10.9	25.6	2.0	307.4			100	
BDE-99	28.2	4.7	16.2	0.8	183.2	60.2	43.0	100	7.3
BDE-47	7.1	2.0	3.9	0.5	52.5	15.2	18.2	100	6.8
BDE-100	5.9	1.1	3.3	0.2	40.3	12.6	9.9	100	7.2
BDE-153	2.2	0.4	1.2	<0.2	13.4	4.7	4.0	57.1	7.9
BDE-154	1.3	0.3	0.7	<0.2	8.7	2.8	2.9	35.7	7.8
BDE-209	1.0	0.7	0.2	<0.2	2.3	2.2	6.6	78.6	10.3

[a] Log octanol–water coefficient values from Braekevelt et al. (2003).

present starling study, the lack of a strong correlation between *p,p′*-DDE and ΣPBDEs or ΣPCBs, and the positive correlation between ΣPBDEs and ΣPCBs, may also be linked to differences in the historical spatial patterns of use among the contaminant classes. The variation between contaminant class concentrations in eggs could also be a result of differential maternal transfer to eggs of each compound (Verreault et al., 2006). The strength of the contaminant class correlations may also be low due to small sample sizes.

Comparing organohalogen concentrations in starling eggs from our study to reported values for other passerines in British Columbia, starling eggs had significantly lower mean *p,p′*-DDE concentrations (geometric mean [GM] = 36.53 ng/g ww) than American robin (*Turdus migratorius*) eggs in both agricultural sites in the Okanagan Valley (GM = 39,300 ng/g ww), and in non-agricultural sites in the same geographic region as our study (GM = 1100 ng/g ww; Gill et al., 2003), but somewhat higher concentrations than American dipper (*Cinclus mexicanus*) eggs in southern BC rivers (GM = 9.4 ng/g ww; Morrissey et al., 2010). DDT has been banned in North America since the 1970s, and we did not detect the parent compound, *p,p′*-DDT, in any of the starling eggs. The only form detected was the principle metabolite *p,p′*-DDE, which indicates it is from a historic origin rather than a recent release. It is unlikely that *p,p′*-DDE concentrations at our study site would negatively affect productivity in starlings, as the effect level in the brown pelican (*Pelecanus occidentalis*), which is reportedly the most sensitive avian species to DDT, was 3 μg *p,p′*-DDE/g ww in the eggs (Blus, 2011), and in American robins, reproductive success was not affected by mean ΣDDT concentrations of 48 μg/g ww (Gill et al., 2003). Concentrations of other OC pesticides in starling eggs in our study (~0.2 to 2 ng/g ww) were also well below those reported to cause adverse effects in birds (Elliott and Bishop, 2011).

ΣPCB concentrations in our study (GM = 3.58 ng/g ww) were surprisingly low, even less than in robin eggs from agricultural (GM = 49.2 ng/g ww) and non-agricultural (GM = 79.5 ng/g ww; Gill et al., 2003) areas, as well as dipper eggs in river basins (GM = 21.2 ng/g ww; Morrissey et al., 2010) in British Columbia. Mean ΣPCBs in starling eggs from a landfill site ~40 km to the west of our study area (1200 ng/g lw, 6.1% lipid, equivalent to 73.2 ng/g ww) were also significantly greater than those encountered here (Eens et al., 2013). These low PCB concentrations may indicate that our site is particularly removed from industrial inputs. Because contaminants can be variable among individuals within a site, it is also possible that there were individuals with higher concentrations of PCBs that we did not detect due to our small sample size. The main PCB congeners detected in starling eggs at our site were all major components of commercially used PCB mixtures. CB-138 and -153 were major congeners in Aroclors 1254, 1260 and 1262, CB-187 and -180 were major congeners in Aroclors 1260 and 1262, and CB-146 was a major congener in Aroclor 1260 (Frame, 1997). Even though starlings appear to be among the more PCB or dioxin sensitive species (Farmahin et al., 2013), it is not likely that ΣPCB concentrations found in starling eggs would have effects on productivity, as they were far below the suggested critical threshold for behavioral effects of 1 to 30 μg ΣPCB s/g ww in eggs (Harris and Elliott, 2011).

Although our study site covered a relatively small geographic area (ca. 3.6 ha), there was very large variability in PBDE burden in eggs from different nests (2 to 307 ng/g ww). This variation could be due to differences in diet or feeding locations among individual mothers, both during the breeding season and the overwintering period. Breeding starlings typically forage in patches within 500 m of the nest and switch patches regularly (Feare, 1984; Tinbergen, 1981). During the winter, starlings roost communally overnight, and then individuals disperse to their feeding areas during the day (Feare, 1984). It is possible that the individuals with high egg ΣPBDEs fed in patches with a source of PBDE contamination. Further investigation into breeding season and overwintering diet and foraging behavior may help explain the source of inter-individual variation in contaminant loads. Inter-individual variation in egg concentrations of persistent contaminants could also be related to the age of the mothers, with older individuals potentially accumulating greater body burdens. For example, in white-tailed eagles (*Haliaeetus albicilla*), high inter-individual variation in organohalogen contaminant loads was largely explained by age differences, with significantly higher concentrations in adults than in juveniles (Jaspers et al., 2013). Similarly, ΣPBDE concentrations in Eurasian sparrowhawks (*Accipiter nisus*) were greater in adults than in juveniles (Crosse et al., 2013). In this study we did not know the age of the maternal starlings, but longer term datasets where the age of the individuals is known would be valuable for investigating effects of age on variation in contaminant burdens.

At the site level, the median ΣPBDEs in our starling eggs (6.6 ng/g ww) were comparable to other rural sites across Canada that were ~40 km from urban centers (median ΣPBDEs ranged from 6.7 to 30.4 ng/g ww), and were lower than industrial (median from 14.6 to 104 ng/g ww) or landfill sites (median from 27.7 to 268.2 ng/g ww) (Chen et al., 2013). Starling eggs at our study site had similar ΣPBDE concentrations (GM = 10.9 ng/g ww) compared to American dipper eggs in British Columbia (GM = 14.1 ng/g ww; Morrissey et al., 2010), and their ΣPBDE concentrations fell within the range of ΣPBDE values reported for passerines outside of British Columbia. Our starling eggs had approximately 10 times more PBDEs than the highest mean values reported for great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) across Europe (Van den Steen et al., 2009b, 2010b). They had similar ΣPBDE levels to cliff swallow (*Petrochelidon pyrrhonota*) carcasses in agricultural areas of Texas (GM = 18 ng/g ww), but significantly lower concentrations than cliff swallows in heavily industrialized areas (GM = 258 ng/g ww and 196 ng/g ww; Mora et al., 2012). In passerine birds in China, the highest levels of ΣPBDEs were near electrical-waste sites, with concentrations in pectoral muscle up to 15,000 ng/g lipid weight (lw), followed by urban, then suburban, then rural sites (Sun et al., 2012). Our concentrations in European starling eggs (median 186.5 ng/g lw) were the most similar to concentrations measured at suburban locations in China (median values from 160 to 350 ng/g lw). Overall in passerines, the concentration of ΣPBDEs tends to reflect the type of land use.

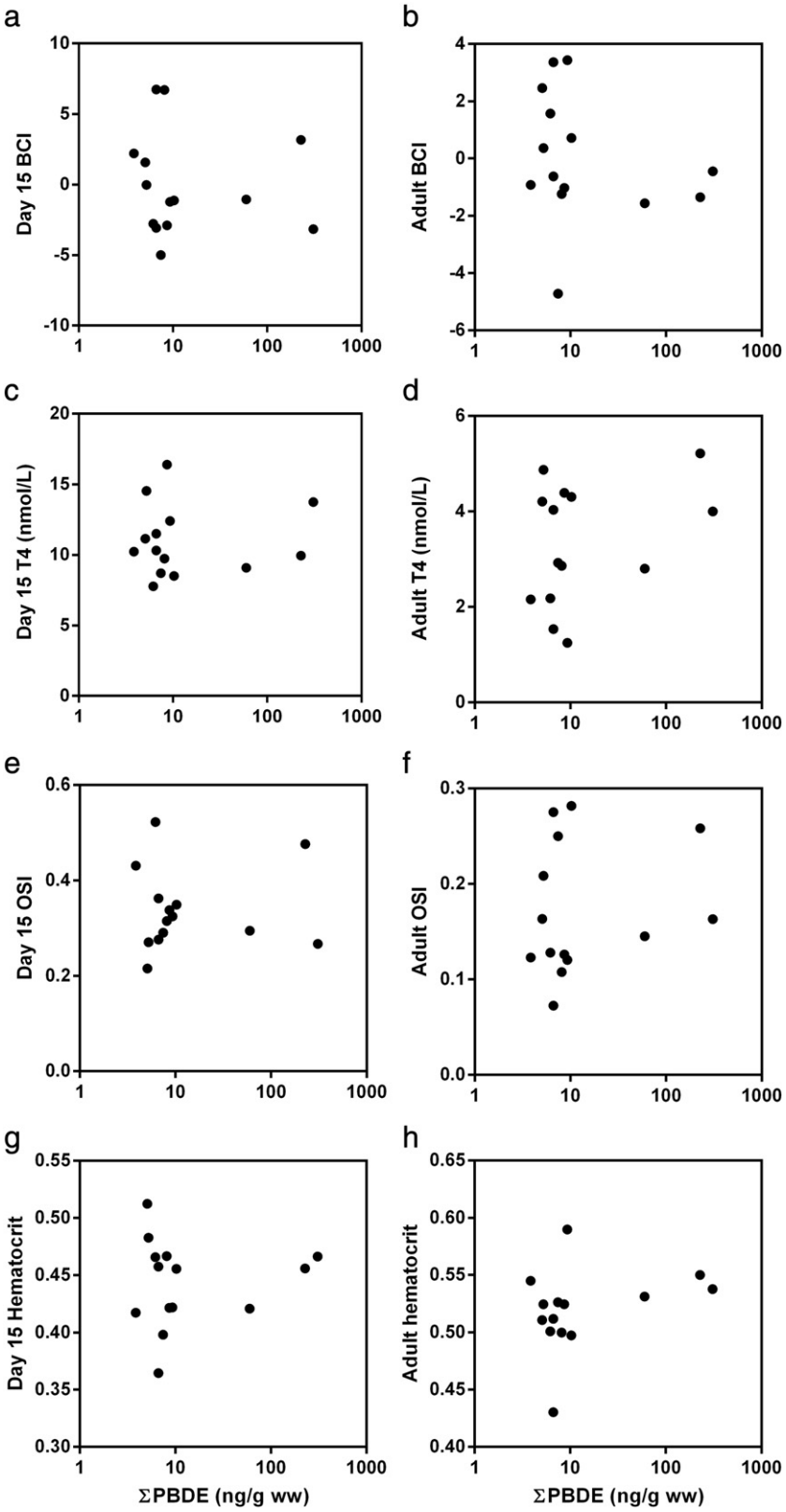

Fig. 1. Relationship between ΣPBDEs in European starling eggs and (a) BCI of day 15 nestlings, (b) BCI of adult females, (c) plasma T4 at day 15, (d) plasma T4 of adult females, (e) OSI at day 15, (f) OSI of adult females, (g) hematocrit at day 15, and (h) hematocrit of adult females. BCI = body condition index, T4 = plasma thyroxine, OSI = oxidative status index.

Stable isotope studies have shown that both trophic level (Elliott et al., 2010; Sun et al., 2012) and habitat type (Elliott et al., 2009; Newsome et al., 2010) can influence PBDE accumulation. Starlings primarily feed on soil invertebrates and are a mid-trophic level species in terrestrial food chains. Concentrations of ΣPBDEs in our starling eggs (AM = 46.9 ng/g ww, 3.6% lipid) fell within the range of values reported for top predators in British Columbia. Arithmetic mean ΣPBDEs were from 1.78 to 8.49 ng/g ww in bald eagle nestling (*Haliaeetus leucocephalus*) plasma (~1% lipid) on the west coast (McKinney et al., 2006), 47.9 ng/g ww in great blue heron (*Ardea herodias*) eggs (avg.

6.15% lipid) on Vancouver Island, 62.5 ng/g ww in double-crested cormorant (*P. auritus*) eggs (avg. 6.58% lipid) on the west coast, 185 ng/g ww in osprey (*Pandion haliaetus*) eggs (avg. 4.67% lipid) from southern river systems, and 455 ng/g ww in heron eggs (avg. 5.92% lipid) on the southwest coast (Elliott et al., 2005). It appears as though that the level of urbanization or industrialization and resulting contaminant concentrations in the local environment can have a large influence on the accumulation of PBDEs, as birds within similar trophic levels can have a broad range of PBDE burdens, and PBDE burdens overlap across trophic levels.

Although the lower brominated BDEs were regulated in Canada in 2006, they still made up over 95% of the ΣPBDEs in starling eggs. The PBDE congener profile in starling eggs (BDE-99 > -47 > -100 > -153 > -154) matches the profile of DE-71, which was the major penta-BDE technical flame-retardant mixture manufactured in North America (La Guardia et al., 2006). Low levels of BDE-209, which is the main component of the deca-BDE technical mixture (Saytex 102E), were also detected in most (78.6%) of the starling eggs. The presence of BDE-209 in starling eggs likely indicates recent exposure, as it has been shown to have a relatively short half-life in both European starlings (13 days; Van den Steen et al., 2007) and American kestrels (*Falco sparverius*) (14 days; Letcher et al., 2013), with evidence of metabolic debromination in both species. In the present study, the major components of the octa-BDE technical mixture (i.e. BDE-183, -197, -207, -196) made up less than 1% of the ΣPBDEs in starling eggs. PBDE congener profiles in other bird species in British Columbia are also generally dominated by the penta-BDEs (e.g. Elliott et al., 2005; McKinney et al., 2006; Morrissey et al., 2010; Wilson et al., 2010).

Despite the marked inter-individual variability in egg PBDE levels the concentrations of ΣPBDEs in starling eggs were not related to reproductive success or any measures of condition in the mothers or offspring of the corresponding nests (i.e. from the remaining non-sampled eggs). This is not surprising as concentrations in starling eggs were generally lower than those reported to cause effects in birds. To further our understanding of the effects of PBDEs in passerines, future studies relating individual variation in PBDE concentrations to individual measures of reproduction and condition should be conducted with European starlings in sites that have demonstrated very high levels of PBDEs in terrestrial passerines (i.e. landfill sites). In free-living osprey (*P. haliaetus*), effects on productivity were not observed until ΣPBDEs were greater than 1000 ng/g ww in eggs (Henny et al., 2009). In bird eggs exposed to a penta-BDE mixture through egg injection, the lowest concentration to affect hatching success was 1.8 μg/g ww in American kestrels, and doses up to 20 μg/g ww had no effect on embryo survival endpoints in chicken (*Gallus gallus*) or mallard (*Anas platyrhynchos*) eggs (McKernan et al., 2009). European starlings that were exposed to a penta-BDE mixture through subcutaneous implants (~1740 ng/g bw) and produced eggs with ΣPBDEs from 130 to 220 ng/g ww showed some effects on egg size, but there were otherwise minimal effects on reproductive, endocrine disrupting, hematological and biochemical endpoints (Van den Steen et al., 2009a, 2010a). Zebra finches (*Taeniopygia guttata*) exposed to BDE-99 via egg injection showed reduced clutch sizes at concentrations as low as 10 ng/g ww (Winter et al., 2013), which is similar to the ΣPBDE concentrations seen in starling eggs. However, the clutch size effects in zebra finches could not be seen until in ovo exposed birds reached reproductive maturity, and to assess whether such effects occur in the field, long term studies that link in ovo exposure to adult productivity are needed.

In conclusion, we found that European starlings in a rural agricultural area of British Columbia are being exposed to PBDEs, PCBs, DDTs, and other OCs at levels below those of concern. European starlings are found globally, and are exposed to a broad range of contaminant concentrations (Eens et al., 2013). By collecting a single egg per nest we were able to assess the inter-individual variation in contaminants among nests, and assess whether PBDE concentrations in eggs were related to the productivity and condition of the corresponding nests. While we did not see effects in birds at a rural agricultural site with relatively low contaminant burdens, the European starling could potentially be a useful species for linking contaminant concentrations with biological effects in terrestrial passerines at sites with higher levels of pollution.

Acknowledgments

The authors thank the Ydenbergs for the use of their property, S. Parker for field assistance, and L. Periard (Organic Contaminants Research Laboratory, National Wildlife Research Centre) for contaminant analysis. This work was primarily supported by the Chemicals Management Plan of Environment Canada, and was also funded by a Natural Sciences and Engineering Research Council Postgraduate Scholarship to M.L.E.

References

Arenal CA, Halbrook RS, Woodruff M. European starling (*Sturnus vulgaris*): avian model and monitor of polychlorinated biphenyl contamination at a Superfund site in southern Illinois, USA. Environ Toxicol Chem 2004;23:93–104.
Blus L. DDT, DDD and DDE in birds. Environmental contaminants in biota. CRC Press; 2011425–44.
Braekevelt E, Tittlemier SA, Tomy GT. Direct measurement of octanol-water partition coefficients of some environmentally relevant brominated diphenyl ether congeners. Chemosphere 2003;51:563–7. http://dx.doi.org/10.1016/s0045-6535(02)00841-x.
Braune BM, Mallory ML, Gilchrist HG, Letcher RJ, Drouillard KG. Levels and trends of organochlorines and brominated flame retardants in Ivory Gull eggs from the Canadian Arctic, 1976 to 2004. Sci Total Environ 2007;378:403–17.
Canada Gazette. Part II. Polybrominated diphenyl ether regulations. 2008;142:1663–82.
Cesh LS, Elliott KH, Quade S, McKinney MA, Maisoneuve F, Garcelon DK, et al. Polyhalogenated aromatic hydrocarbons and metabolites: relation to circulating thyroid hormone and retinol in nestling bald eagles (*Haliaeetus leucocephalus*). Environ Toxicol Chem 2010;29:1301–10.
Chen D, Hale RC. A global review of polybrominated diphenyl ether flame retardant contamination in birds. Environ Int 2010;36:800–11.
Chen D, Martin P, Burgess NM, Champoux L, Elliott JE, Forsyth DJ, et al. European Starlings (*Sturnus vulgaris*) Suggest That Landfills Are an Important Source of Bioaccumulative Flame Retardants to Canadian Terrestrial Ecosystems. Environmental Science & Technology 2013;47:12238–47. http://dx.doi.org/10.1021/es403383e.
Crosse JD, Shore RF, Jones KC, Pereira MG. Key factors affecting liver PBDE concentrations in sparrowhawks (*Accipiter nisus*). Environ Pollut 2013;177:171–6.
Custer TW, Pendleton G, Ohlendorf HM. Within-clutch and among-clutch variation of organochlorine residues in eggs of black-growned night-herons. Environ Monit Assess 1990;15:83–9.
de Wit CA. An overview of brominated flame retardants in the environment. Chemosphere 2002;46:583–624.
Eens M, Jaspers VLB, Van den Steen E, Bateson M, Carere C, Clergeau P, et al. Can starling eggs be useful as a biomonitoring tool to study organohalogenated contaminants on a worldwide scale? Environ Int 2013;51:141–9.
Elliott JE, Bishop C. Cyclodiene and other organochlorine pesticides in birds. Environmental contaminants in biota. CRC Press; 2011447–75.
Elliott JE, Wilson LK, Wakeford B. Polybrominated diphenyl ether trends in eggs of marine and freshwater birds from British Columbia, Canada, 1979–2002. Environ Sci Technol 2005;39:5584–91.
Elliott KH, Cesh LS, Dooley JA, Letcher RJ, Elliott JE. PCBs and DDE, but not PBDEs, increase with trophic level and marine input in nestling bald eagles. Sci Total Environ 2009;407:3867–75.
Elliott JE, Wilson LK, Drouillard K. Accumulation of PBDEs and legacy POPs in urban raptors from British Columbia, Canada, 1999–2009. Society of Environmental Toxicology and Chemistry North America 31st Annual Meeting, Portland, OR; 2010.
Eng ML, Williams TD, Elliott JE. Early exposure to BDE-99: an assessment of effects on physiology, growth, and reproduction in the zebra finch. Environ Pollut 2013;178:343–9.
Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277–85.
Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103–11.
Farmahin R, Manning GE, Crump D, Wu DM, Mundy LJ, Jones SP, et al. Amino acid sequence of the ligand-binding domain of the aryl hydrocarbon receptor 1 predicts sensitivity of wild birds to effects of dioxin-like compounds. Toxicol Sci 2013;131:139–52.
Feare CJ. The starling. New York, N.Y.: Oxford University Press; 1984.
Fernie KJ, Shutt JL, Mayne G, Hoffman D, Letcher RJ, Drouillard KG, et al. Exposure to polybrominated diphenyl ethers (PBDEs): changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). Toxicol Sci 2005;88:375–83.
Frame GM. A collaborative study of 209 PCB congeners and 6 Aroclors on 20 different HRGC columns. 2. Semi-quantitative Aroclor congener distributions. Fresenius J Anal Chem 1997;357:714–22.

Gauthier LT, Hebert CE, Weseloh DVC, Letcher RJ. Dramatic changes in the temporal trends of polybrominated diphenyl ethers (PBDEs) in herring gull eggs from the Laurentian Great Lakes: 1982–2006. Environ Sci Technol 2008;42:1524–30.
Gill H, Wilson LK, Cheng KM, Elliott JE. An assessment of DDT and other chlorinated compounds and the reproductive success of American robins (*Turdus migratorius*) breeding in fruit orchards. Ecotoxicology 2003;12:113–23.
Harris M, Elliott JE. Effects of polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans and polybrominated diphenyl ethers in wild birds. Environmental Contaminants in Biota. CRC Press; 2011477–528.
Henny CJ, Kaiser JL, Grove RA, Johnson BL, Letcher RJ. Polybrominated diphenyl ether flame retardants in eggs may reduce reproductive success of ospreys in Oregon and Washington, USA. Ecotoxicology 2009;18:802–13.
Jaspers VLB, Sonne C, Soler-Rodriguez F, Boertmann D, Dietz R, Eens M, et al. Persistent organic pollutants and methoxylated polybrominated diphenyl ethers in different tissues of white-tailed eagles (*Haliaeetus albicilla*) from West Greenland. Environ Pollut 2013;175:137–46.
La Guardia MJ, Hale RC, Harvey E. Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. Environ Sci Technol 2006;40:6247–54.
Law RJ, Alaee M, Allchin CR, Boon JP, Lebeuf M, Lepom P, et al. Levels and trends of polybrominated diphenylethers and other brominated flame retardants in wildlife. Environ Int 2003;29:757–70.
Letcher RJ, Marteinson SC, Fernie KJ. Dietary exposure of American kestrels (*Falco sparverius*) to decabromodiphenyl ether (BDE-209) flame retardant: Uptake, distribution, debromination and cytochrome P450 enzyme induction. Environment International 2013;63:182–90. http://dx.doi.org/10.1016/j.envint.2013.11.010.
McKernan MA, Rattner BA, Hale RC, Ottinger MA. Toxicity of polybrominated diphenyl ethers (DE-71) in chicken (*Gallus gallus*), mallard (*Anas platyrhynchos*), and American kestrel (*Falco sparverius*) embryos and hatchlings. Environ Toxicol Chem 2009;28:1007–17.
McKinney MA, Cesh LS, Elliott JE, Williams TD, Garcelon DK, Letcher RJ. Brominated flame retardants and halogenated phenolic compounds in North American west coast bald eaglet (*Haliaeetus leucocephalus*) plasma. Environ Sci Technol 2006;40:6275–81.
Mora MA, Sericano JL, Baxter C. Swallows as indicators of environmental pollution of the Rio Grande/Rio Bravo Basin: are persistent organic pollutants a concern? Arch Environ Contam Toxicol 2012;62:512–8.
Morrissey CA, Elliott JE, Ormerod SJ. Local to continental influences on nutrient and contaminant sources to river birds. Environ Sci Technol 2010;44:1860–7.
Newsome SD, Park J-S, Henry BW, Holden A, Fogel ML, Linthicum J, et al. Polybrominated diphenyl ether (PBDE) levels in peregrine falcon (*Falco peregrinus*) eggs from California correlate with diet and human population density. Environ Sci Technol 2010;44:5248–55.
SAS Institute. SAS version 9.1. Cary, North Carolina, USA: SAS Institute; 2003.
Schulte-Hostedde AI, Zinner B, Millar JS, Hickling GJ. Restitution of mass-size residuals: validating body condition indices. Ecology 2005;86:155–63.
Sun YX, Luo XJ, Mo L, Zhang Q, Wu JP, Chen SJ, et al. Brominated flame retardants in three terrestrial passerine birds from South China: geographical pattern and implication for potential sources. Environ Pollut 2012;162:381–8.
Tinbergen JM. Foraging decisions in starlings (*Sturnus vulgaris* L.). Ardea 1981;69:1–67.
UNEP. Draft revised guidance on the global monitoring plan for persistent organic pollutants, UNEP/POPS/COP.5/INF/27. Geneva, Switzerland: United Nations Environment Programme, UNEP Chemicals; 2011.
Van den Steen E, Dauwe T, Covaci A, Jaspers VLB, Pinxten R, Eens M. Within- and among-clutch variation of organohalogenated contaminants in eggs of great tits (*Parus major*). Environ Pollut 2006;144:355–9.
Van den Steen E, Covaci A, Jaspers VLB, Dauwe T, Voorspoels S, Eens M, et al. Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (*Sturnus vulgaris*). Environ Pollut 2007;148:648–53.
Van den Steen E, Jaspers VLB, Covaci A, Dauwe T, Pinxten R, Neels H, et al. Variation, levels and profiles of organochlorines and brominated flame retardants in great tit (*Parus major*) eggs from different types of sampling locations in Flanders (Belgium). Environ Int 2008;34:155–61.
Van den Steen E, Eens M, Covaci A, Dirtu AC, Jaspers VLB, Neels H, et al. An exposure study with polybrominated diphenyl ethers (PBDEs) in female European starlings (*Sturnus vulgaris*): toxicokinetics and reproductive effects. Environ Pollut 2009a;157:430–6.
Van den Steen E, Pinxten R, Jaspers VLB, CovaCi A, Barba E, Carere C, et al. Brominated flame retardants and organochlorines in the European environment using great tit eggs as a biomonitoring tool. Environ Int 2009b;35:310–7.
Van den Steen E, Eens M, Geens A, Covaci A, Darras VM, Pinxten R. Endocrine disrupting, haematological and biochemical effects of polybrominated diphenyl ethers in a terrestrial songbird, the European starling (*Sturnus vulgaris*). Sci Total Environ 2010a;408:6142–7.
Van den Steen E, Pinxten R, Covaci A, Carere C, Eeva T, Heeb P, et al. The use of blue tit eggs as a biomonitoring tool for organohalogenated pollutants in the European environment. Sci Total Environ 2010b;408:1451–7.
Verreault J, Villa RA, Gabrielsen GW, Skaare JU, Letcher RJ. Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. Environ Pollut 2006;144:1053–60.
Verreault J, Bech C, Letcher RJ, Ropstad E, Dahl E, Gabrielsen GW. Organohalogen contamination in breeding glaucous gulls from the Norwegian Arctic: associations with basal metabolism and circulating thyroid hormones. Environ Pollut 2007;145:138–45.
Wilson LK, Harris ML, Trudeau S, Ikonomou MG, Elliott JE. Properties of blood, porphyrins, and exposure to legacy and emerging persistent organic pollutants in surf scoters (*Melanitta perspicillata*) overwintering on the south coast of British Columbia, Canada. Arch Environ Contam Toxicol 2010;59:322–33.
Winter V, Williams TD, Elliott JE. A three-generational study of in ovo exposure to PBDE-99 in the zebra finch. Environ Toxicol Chem 2013;32:562–8.