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Birds and flame retardants: A review of the toxic effects on birds of historical and novel flame retardants

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ABSTRACT

Flame retardants (FRs) are a diverse group of chemicals, many of which persist in the environment and bioaccumulate in biota. Although some FRs have been withdrawn from manufacturing and commerce (e.g., legacy FRs), many continue to be detected in the environment; moreover, their replacements and/or other novel FRs are also detected in biota. Here, we review and summarize the literature on the toxic effects of various FRs on birds. Birds integrate chemical information (exposure, effects) across space and time, making them ideal sentinels of environmental contamination. Following an adverse outcome pathway (AOP) approach, we synthesized information on 8 of the most commonly reported endpoints in avian FR toxicity research: molecular measures, thyroid-related measures, steroids, retinol, brain anatomy, behaviour, growth and development, and reproduction. We then identified which of these endpoints appear more/most sensitive to FR exposure, as determined by the frequency of significant effects across avian studies. The avian thyroid system, largely characterized by inconsistent changes in circulating thyroid hormones that were the only measure in many such studies, appears to be moderately sensitive to FR exposure relative to the other endpoints; circulating thyroid hormones, after reproductive measures, being the most frequently examined endpoint. A more comprehensive examination with concurrent measurements of multiple thyroid endpoints (e.g., thyroid gland, deiodinase enzymes) is recommended for future studies to more fully understand potential avian thyroid toxicity of FRs. More research is required to determine the effects of various FRs on avian retinol concentrations, inconsistently sensitive across species, and to concurrently assess multiple steroid hormones. Behaviour related to courtship and reproduction was the most sensitive of all selected endpoints, with significant effects recorded in every study. Among domesticated species (Galliformes), raptors (Accipitriformes and Falconiformes), songbirds (Passeriformes), and other species of birds (e.g. gulls), raptors seem to be the most sensitive to FR exposure across these measurements. We recommend that future avian research connect biochemical disruptions and changes in the brain to ecologically relevant endpoints, such as behaviour and reproduction. Moreover, connecting *in vivo* endpoints with molecular endpoints for non-domesticated avian species is also highly important, and essential to linking FR exposure with reduced fitness and population-level effects.

1. Introduction

The current extent of chemical pollution has led some authors (e.g., Rockström et al., 2009) to conclude that the resulting damage to the environment is rapidly becoming irreversible. There are tens of thousands of human-made compounds in commerce and identifying those compounds that are most hazardous to the environment is critical. Some of the first chemical pollutants were organochlorine pesticides, such as the "Dirty Dozen", and these were eventually regulated by the Stockholm Convention (http://chm.pops.int/). Flame retardants (FRs) are a diverse group of chemicals, many of which are added to plastics, textiles (e.g. furniture) and surface finishes (e.g. electronics), and used to inhibit or delay the spread of fire (Bergman et al., 2012; de Wit, 2002; van der Veen and de Boer, 2012). Moreover, additive FRs diffuse out of the polymers used in products and are released into the environment. Although FRs degrade during fire to release halogens, in turn quenching the fire, FRs are environmentally stable and hence persist. Due to the persistent nature of many FRs and their propensity to accumulate in biota, understanding the toxicity of flame retardants to wildlife is a critical

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question in environmental research and policy.

There are several types of FRs: brominated FRs, chlorinated FRs, and organophosphate FRs or esters (OPEs). Some FRs are legacy FRs, whose production and use are regulated, banned or have been voluntarily withdrawn, but those compounds have been replaced with new compounds. Many of the legacy FRs contain bromines, which as halogens, share many toxic properties (Darnerud, 2003) with chlorinated compounds such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyl (PCB), and hexachlorobenzene (HCB), all chemicals listed under the "Dirty Dozen" (http://chm.pops.int/). In particular, polybrominated diphenyl ethers (PBDEs), especially the tetra- and octa-BDEs, increased exponentially in the environment prior to their phase-out in the mid-2000s (Elliott et al., 2005; Lindberg et al., 2004), and were eventually added to the Stockholm Convention (http://chm.pops.int/). PBDE levels have declined since many were phased out, but high levels of these bioaccumulative compounds still remain in top predators (Elliott et al., 2015; Fernie and Letcher, 2010; Guerra et al., 2011, 2012; Law et al., 2014). More recently, other FRs (e.g., hexabromocyclododecane (HBCD)) have been added to the Stockholm Convention, including the commercial mixture, deca-BDE, and short-chained chlorinated paraffins, that were listed in 2016 (http://chm.pops.int/).

As a result of the withdrawal of PBDEs from the market, alternative brominated FRs such as HBCD and non-brominated compounds such as OPEs were introduced and have increased in use. These alternative FRs have also been detected in increasing concentrations in the environment, with some observed at high concentrations in avian predators at high trophic positions in aquatic and terrestrial food webs (Chen et al., 2012a; Johansson et al., 2009; Lindberg et al., 2004). While some alternative FRs were subsequently assessed and regulated under the Stockholm Convention (e.g., HBCD, short-chained chlorinated paraffins), some remain in use, like a number of the OPEs. Even though several OPEs do not appear to bioaccumulate to the same degree as PBDEs, they were recently identified as priority chemicals for risk assessment by Environment and Climate Change Canada (formerly Environment Canada), in part because of their presence in biota (Greaves et al., 2014). Clearly, the toxicity of these novel compounds and other FRs is important to establish.

Birds are often used as sentinels for environmental contamination. Many of the trends in FR levels are derived from avian research and monitoring programs. Birds, especially those feeding at a high trophic position in the food chain, such as birds of prey, seabirds, and gulls, are particularly useful sentinels for monitoring flame retardants because 1) they can accumulate high levels of contaminants, 2) they integrate signals over large spatial and temporal scales, and 3) they often return to a central place (e.g., a nest site or breeding colony) where they may be accessed for sample collection and observed for some reproductive parameters. Because birds are often used in monitoring programs (Chen and Hale, 2010; Chen et al., 2012b), and have relatively high levels of pollutants making them particularly susceptible to potential effects of the chemicals, understanding of the toxicity of FRs and other chemicals in birds is strongly needed.

The FRs included in this review have been detected in the tissues of wild birds. Birds are used throughout the world for characterizing and monitoring FR levels (Abbasi et al., 2016; Chen et al., 2012a; Eens et al., 2013; Eulaers et al., 2014; Gómez-Ramírez et al., 2014;Jaspers et al. 2006). In a global review on PBDE contamination in birds, Chen and Hale (2010) reported that PBDE levels in terrestrial birds, in particular the chemical mixture deca-BDE which predominantly contains BDE-209, were highest in North America and China. In general, terrestrial birds had higher deca-BDE concentrations than aquatic birds (Chen and Hale, 2010), and the highest PBDE concentration reported in any wild bird was from a terrestrial apex predator, a Cooper's hawk (*Accipiter cooperii*) (Elliott et al., 2015). Both legacy and novel brominated FRs have also been reported in another terrestrial apex predator, the peregrine falcon (*Falco peregrinus*), in which the highest concentration of HBCD for any biota was reported in a peregrine egg (Guerra et al., 2012). Finally, novel OPEs have been detected in herring gull *(Larus argentatus)* eggs from the Great Lakes in North America (Chen et al., 2012a; Greaves and Letcher, 2014). In sum, the detection of a variety of FRs in multiple species of wild birds provides the ecological context for captive toxicity studies and the need for a review of the toxicity of FRs in birds.

Here we provide a critical review of the *in vitro* and *in vivo* toxic effects of flame retardants on birds reported to date (2016). Our goal is to use the Adverse Outcome Pathway (AOP) framework and apply it to avian toxicity research with flame retardants generally. The AOP is a tool that integrates knowledge relating to the links between a molecular initiating event, such as exposure to a chemical, and a chain of events at increasing levels of biological organization (Ankley et al., 2010). Specifically, we examine which endpoints showed consistent effects across chemicals and species in relation to the birds' exposure to flame retardants. We consider the most commonly reported endpoints, from molecular and hormone systems through behaviour to reproduction. Because of wide differences in methodologies, such as dosage levels and timing, we cannot use a strict meta-analysis. Rather, we use a weight of evidence approach where we calculate the proportion of studies reporting at least one significant effect for each endpoint.

2. Methods

2.1. Data search methods

We searched initially for the following keywords in Web of Science: 'avian' or 'bird' and 'flame retardant'. This initial search allowed us to determine the most commonly reported endpoints. We then conducted subsequent searches with 'avian' or 'bird' and 'flame retardant' and each endpoint of interest. References from the publications collected from these searches were also collected until we were satisfied that all relevant references were included. In addition to experimental studies, we included field studies that examined correlations between tissue or plasma concentrations of flame retardants and endpoints of interest. Although we did not set a limitation to how far back we went in time, we only collected articles that were available electronically via the McGill University Web of Science portal. We included a total of 61 studies in our review.

2.2. Types of flame retardants

We included in our review, FRs that have been phased out but are still detected in the environment (e.g. PBDEs), FRs that are currently being phased out (HBCD), and FRs that are currently in use, such as 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane (DBE-DBCH; formerly abbreviated as TBECH) and tetrabromobisphenol A (TBBPA). Although most of the studies in this review focused on brominated flame retardants (BFRs), some focused on OPEs such as tris (1,3-dichloro-2-propyl) phosphate (TDCIPP), tris(2-chloroisopropyl) phosphate (TCIPP), tris(2-butoxyethyl) phosphate (TBOEP), tris(2-chloroethyl) phosphate (TCEP), and tris(methylphenyl) phosphate (TMPP). Other FRs included in the review but to a lesser degree than those described above are: Dechlorane Plus (DP; also known as bis(hexachlorocyclopentadieno) cyclooctane), hexachlorocyclopentenyldibromocyclooctane (HCDBCO or DBHCTD), bis(2-ethlylhexyl)tetrabromophthalate (BEHTBP, now BEH-TEBP), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenyl ethane (DBDPE), tetrabromobisphenol A bis(2,3-dibromopropyl ether) (TBBPA-BDBPE), tris(4-*tert*-butylphenyl) phosphate (TBPP), tris(2-ethylhexyl) phosphate (TEHP), tris(2-butoxyethyl) phosphate (TBEP), melamine (Mel), chlorendic acid (CA), bis(2-ethylhexyl) phosphate (HDEHP), vinyl bromide (VB), tricresyl phosphate (TCP), allyl 2,4,6-tribromophenyl ether (ATE), 2,2-bis(bromomethyl)-1,3-propanediol (BBMP), and tris(2,3-dibromopropyl) isocyanurate (TBC). In sum, we included in our review,

studies that examined the effects of any type of FR (legacy or novel) on birds in the context of the following endpoints, which are explained in detail below: measures of the thyroid system, concentrations of steroids, retinol, brain anatomy, behaviour, growth and development, and reproduction.

2.3. Species-specific effects

Each species is likely to be impacted differently by FRs due to species-specific differences in genetics, physiology, ecology, as well as differences in their environment. A classic example of species-specific effects in toxicology is the effect of dioxin-like compounds on the aryl hydrocarbon (Ah) receptor, a major mechanism for the toxicity of dioxin and dioxin-like compounds (Head et al., 2008). Species vary almost 1000-fold in the sensitivity of their Ah receptor to dioxin-like toxicity due to variations in a few key amino acids (Head et al., 2008); that variation has no phylogenetic basis, so that two species in the same family can have very different sensitivities to a chemical (Head et al., 2008). Given that we have no clear mechanism for FR toxicity, and there are many endpoints and types of FR chemicals involved, such species-specific variation is difficult to establish for FRs, although it may exist. For instance, American kestrels (*Falco sparverius*) experienced a negative impact of PBDEs on hatching success whereas chickens (*Gallus gallus domesticus*) and mallards *(Anas platyrhynchos)* did not, within the same study (McKernan et al., 2009). Although there are likely to be differences in sensitivity among species to the same compound across all of the endpoints we examined, for the purpose of this review, we treat all species as equally sensitive because no study has yet established a mechanism for species-specific variation to FRs. Furthermore, most studies use different dosage regimes or experimental setups meaning that it is not possible to separate species-level effects from differences in experimental setup. Clearly, further study into identifying species-specific variation in sensitivity to FRs, especially in those endpoints that show variation in effects among species, and the mechanisms driving these effects, would be warranted.

Although we treat every species as equally sensitive for the purpose of discussing effects of FRs within each endpoint, we thought it important and interesting to assess the overall species sensitivity across FRs. At the end of the review, we summarize the number of studies in which at least one significant effect was reported for each endpoint for each of the following four avian groups: domesticated gallinaceous species such as chicken and Japanese quail (*Coturnix japonica*; order Galliformes); raptors such as ospreys (*Pandion haliaetus*), bald eagles (*Haliaeetus leucocephalus*), American kestrels, and peregrine falcons (orders Accipitriformes and Falconiformes); songbirds such as European starlings (*Sturnus vulgaris*), tree swallows (*Tachycineta bicolor*), and zebra finches (*Taeniopygia guttata*; order Passeriformes); and all other species of birds included in our review such as glaucous gulls (*Larus hyperboreus*), mallard ducks (*Anas platyrhynchos*), and African penguins (*Spheniscus demersus*). We counted papers with multiple species and/or with multiple chemicals as separate studies in our table. Because the studies addressing transcriptomic and genomic effects of FRs dealt almost exclusively with chickens, we did not include this section in our species comparisons. We discuss the categorical findings of species sensitivity at the end of our review.

2.4. Field versus laboratory studies

Laboratory dosage-exposure studies can carefully control for confounding variables that occur in field situations. For instance, diet, foraging behaviour, and pre-breeding habitat use by birds in the wild are likely to determine exposure and subsequent levels of FRs while at the same time influencing hormone levels, reproductive success, and behaviour of the individual. Thus, we view laboratory studies as important first steps for determining and characterizing the toxicity or "effects" of FRs at specific concentrations, and hence we use the term "effects" only in the context of laboratory studies. However, in many cases the goal is to establish whether effects of a FR are occurring at concentrations to which wild birds are exposed to and/or accumulate in their tissues (e.g., eggs, blood plasma). Moreover, laboratory animals are often fed *ad libitum* and Bustnes et al. (2015) showed that effects of PBDEs were only present in food-stressed birds in the wild because birds fed supplemental food could presumably compensate for the effect of contaminants. For example, in a captive study in which birds were fed *ad libitum*, female American kestrels were able to compensate for their mates' reduced delivery rates of food to their brood (Marteinson et al., 2012a). Thus, it might be expected that wild animals would be more sensitive to possible effects of FRs than well-fed animals in laboratory studies. Nonetheless, because it is difficult to account for confounding effects in field studies, we used the term "correlated" only in the context of field studies. For our endpoint summaries, we combine the results of both laboratory and field studies together to increase sample size, and in these circumstances, we use the word "associated" to refer to either field or laboratory effects.

2.5. Doses

The exposure concentration, or "dose", is one of the most important factors influencing toxicity. Because of the various tissues used to measure the uptake and ensuing FR concentrations as a function of the dosages of chemicals used in studies, and the different methods (delivery and timing) used for dosing, it is difficult to incorporate the effect of varying doses within our analyses. Many of the studies attempted to achieve environmentally-relevant exposure or dosing levels; for species that occupy a higher trophic position (e.g., raptors) than other species (e.g., songbirds), this may mean that these birds may be exposed to and may accumulate higher concentrations of FRs (depending on the physicochemical properties) than other species that occupy a lower trophic position. As such, these differences should be considered especially when interpreting and comparing the effects of FRs across avian species.

2.6. Significance level

Although nearly all studies included in our review set α at 0.05, some set α at 0.1. To ensure consistency between studies, we closely examined all results of interest in detail and only report an effect, a correlation, or an association if $p \leq 0.05$. Nevertheless, we report the pattern of findings for those studies that set α at 0.1 to more fully characterize possible effects and to reduce the chances of mistakenly concluding that a particular FR has no biological effect(s) for that endpoint, when there is.

3. Synthesis of endpoints

3.1. Changes in mRNA expression

The adoption of molecular approaches, such as transcriptomics and genomics, has been one of the major advances in toxicology in the last decade. These techniques hold the promise of replacing hundreds of endpoints with a single microarray. For example, gene expression associated with many of the endpoints discussed in this review could be assessed on hepatocytes *in vitro* in a single experiment. There are particular genes, especially those associated with thyroid function and growth, that are sensitive to a wide array of flame retardants (Crump et al., 2012). However, there is much need for further research and the development of techniques (e.g., RNA sequencing) that would allow for such assessments using non-domesticated, wild avian species that are ecologically relevant or equivalent to those species found in the wild. Indeed, there is also the need to substantiate *in vitro* findings within the context of *in vivo*, whole-animal studies, ideally combining them in

the same study. As noted before, this is especially important given the variation in species-sensitivity to chemicals, including the varying sensitivity of chickens to different chemicals (e.g., highly sensitive to dioxins but less sensitive to PBDEs compared to other species).

We used a different unit of interest in this section relative to the other endpoints in our review. In other sections below, we separated a given study that incorporated two types of FRs, for example, into two "studies" that each examined a single FR. Because a transcriptomic and genomic approach can simultaneously incorporate many more chemicals with a single study (up to 16 chemicals in Table 1b for example), we simplified our syntheses and conclusions in this section by treating each study as a whole and not separating studies based on individual FRs.

Of the 17 studies that examined changes in mRNA expression in relation to *in vitro and in vivo* exposures to FRs, the following five studies utilized avian species that were not chickens as models: Crump et al. (2008a) and Porter et al. (2014) (herring gulls), François et al. (2016) and Técher et al. (2016) (ring-billed gull; *Larus delawarensis*), and Crump et al. (2016) (double-crested cormorant; *Phalacrocorax auritus*) (Table 1b). As such, we recommend that future studies incorporate this promising technique in non-domestic avian species as to more closely reflect potential impacts on wild birds.

We examined changes in mRNA expression of genes *in vivo* and *in vitro* related to other endpoints examined in our review. Specifically, we included effects on genes related to the thyroid system, steroid hormones, the cholinergic system, lipid metabolism, and neurons (e.g., signal transduction and neurite and axonal growth) (Table 1a). Changes in the cholinergic system, lipid metabolism, and neurons, could alter the brain, which is composed primarily of water and lipids, and hence also affect multiple related parameters (e.g., behaviour, growth). In addition, a given gene could simultaneously affect more than one endpoint; for example, the gene insulin growth factor-1 (IGF-1) is involved in both growth and the thyroid system (Table 1a). Of the 13 studies that examined changes in the expression of genes related to the thyroid system in relation to FRs, only one, Crump et al. (2011) failed to find an effect (Table 1b). The study by Crump et al. (2011) used chickens as the model species, and is the only study in our review that examined the effects of the FR, Dechlorane Plus. The other 12 such studies found at least one significant effect (Asnake et al., 2014; Crump et al., 2008b, 2008c, 2012, 2014, 2016; Egloff et al., 2011, 2014; Farhat et al., 2013; François et al., 2016; Porter et al., 2014; Técher et al., 2016), demonstrating that transcription of genes related to the thyroid system seem notably sensitive to exposure to a wide range of FRs (Table 1b). As for studies that incorporated changes in expression of genes related to steroid hormones, three found an effect (Asnake et al., 2014; Farhat et al., 2014; Porter et al., 2014), whereas one did not (Ma et al., 2015). Of the 11 studies that examined changes in expression of genes related to lipid metabolism, the cholinergic system, or signal transduction, neurosteroidogenesis, and neurite and axonal growth, eight found an effect (Crump et al., 2008a, 2008b, 2010, 2012, 2014; Egloff et al., 2014; Farhat et al., 2013; Porter et al., 2014) and three did not (Crump et al., 2008c, 2011; Egloff et al., 2011); determining a pattern(s) of effect(s) among these findings was too difficult because multiple genes/systems and FRs were involved. Finally, of eight studies that examined changes in the expression of IGF-1, a gene related to both the thyroid system and growth, four found that IGF-1 was downregulated in exposed groups (Crump et al., 2010, 2012, 2016; Porter et al., 2014), whereas four found no effect (Crump et al., 2011, 2014; Egloff et al., 2011, 2014). In sum, significant effects were found in the large majority of these studies examining the possible effects of various FRs on the transcription of genes related to the thyroid system (92%; n =13), steroids (75%; n =4), lipid metabolism/cholinergic system/neurons (73%; n =11), and growth (50%; n =8), of birds. As such, each of these endpoints seems sensitive to FRs in the context of *in vitro* and *in vivo* exposure; the possible relationships of these changes in mRNA expression to effects on the same systems, particularly in wild avian species, remain unknown at this time.

Transcriptomic and genomic changes are one of the first stages in the AOP that can result in multiple subsequent effects at higher biological hierarchies, such as hormones, growth, and the brain, for which effects by FRs are presented below.

Table 1a
Details of genes related to our endpoints included in our review.

Gene acronym	Gene full name
Thyroid System	
D1	Type 1 deiodinase
D2	Type 2 deiodinase
D3	Type 3 deiodinase
TTR	Transthyretin
TRα	Thyroid receptor α
TRβ	Thyroid receptor β
TSHR	Thyroid-stimulating hormone receptor
TRIP4	Thyroid hormone receptor interactor 4
NCOA3	Nuclear receptor coactivator 3
TPO	Thyroid peroxidase
OATP1C1	Solute carrier organic anion transporter family, member 1C1
IGF-1	Insulin-like growth factor 1
Sex steroids	
ESR1	Estrogen receptor 1
ESR2	Estrogen receptor 2
VTG	Vitellogenin
VTG2	Vitellogenin 2
ApoII	Apolipoprotein II
Cholinergic system	
nAChR α-7	Neuronal nicotinic acetylcholine receptor alpha-7
Lipid metabolism	
THRSP14-α (Spot 14)	Thyroid hormong-responsive spot 14-α
L-FABP	Liver fatty acid binding protein
ACSL5	Acetyl syntase long-chain family member 5
FABP1	Fatty acid binding protein 1
HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase
SLCO1A2	Solute carrier organic anion transporter family, member 1A2
Signal transduction, neurosteroidogenesis, neurite and axonal growth	
ODZ3; teneurin 3	Odd Oz (odz)/tenascin-major (ten-m) homolog 3
SEB	SET-binding protein
PAG-1	Phospoprotein associated with glycosphingolipid microdomains 1

3.2. Thyroid system

The thyroid hormone pathway is controlled by a cascade of physiological controls within the hypothalamic-pituitary-thyroid (HPT) axis (McNabb, 2007). In the brain, the hypothalamus releases thyroid releasing hormone (TRH), regulated in response to feedback from thyroid receptors and circulating thyroid hormone concentrations, which in turn acts on the pituitary to release thyroid stimulating hormone (TSH). TSH stimulates the thyroid gland to produce and release thyroxine (T_4) into circulation. Deiodinases, largely located in the liver, then convert circulating T_4 into the biologically active triiodothyronine (T_3). T_3 acts on the tissues to increase resting metabolism, among other effects. Individuals with higher resting metabolism are bolder, more aggressive, and more active (Biro and Stamps, 2010). Finally, T_3 and T_4 exert negative feedback control over the hypothalamus. Tight regulation of this endocrine system is critical since it regulates thermoregulation, metabolism, growth, development, behaviour, and in birds, the timing of reproduction and moulting of feathers (McNabb, 2007). Given the limited number of FR-avian studies, for the purposes of this review, we consider T_3 and T_4 independent of whether the study measured total or free forms of these two thyroid hormones.

Table 1b
Flame retardant induced changes in the expression of genes related to other endpoints presented in this review.

Paper	Chemical (s)	Species	Tissue	Dose administered	Concentration in tissues (ng/g ww)	Effects
Asnake et al. (2014) *Env Tox Chem*	DBE-DBCH	Chicken *Gallus gallus domesticus*	Leghorn male hepatoma (LMH) cells	0–10 μM	N/A	ESR1, TRβ, TSHR, TRIP4: ↑ TRα, TTR: ↓ ESR2: –
Crump et al. (2008b) *Tox in Vitro*	DE-71 (penta-BDE mixture; congeners not listed)	Chicken *Gallus gallus domesticus*	Neuronal cell cultures	0.01–300 μM in cell cultures 0.01–3 μM used in RNA isolation	N/A	TTR: ↓ TRα, TRβ: – PAG-1, SEB, ODZ3-teneurin 3: ↑
Crump et al. (2008c) *Environ Sci Technol*	DE-71 (penta-BDE mixture; congeners not listed) BDE-47, BDE-99, BDE-100	Herring gull *Larus argentatus*	Cultured neuronal cells	DE-71: 0.01–300 μM BDE-47, 99, 100: 0.01–3 μM	Adult cortex: ΣBDE47,99,100: 10–96 ΣPBDE[a] 15.44–142.65 Embryonic cortex: ΣBDE47,99,100: 38.19; ΣPBDE[a] 44.31 Neuronal cells from embryonic cell culture: ΣBDE47,99,100: 10.5; ΣPBDE[a] 13.81	DE-71 TTR: ↓ TRα, TRβ, nAChR α–7: – BDE-47, -99, or –100 TTR, TRα, TRβ, nAChR α-7: –
Crump et al. (2008a) *Toxicol Sci*	HBCD DE-71 (penta-BDE mixture; congeners not listed)	Chicken *Gallus gallus domesticus*	Embryonic hepatocytes	HBCD: 0.001–30 μM DE-71: 0.01–300 μM	N/A	HBCD D1, D2, D3: – DE-71 Spot 14, L-FABP, TTR: ↓
Crump et al. (2010) *Toxicol Sci*	HBCD-TM (technical mix; αβγ)	Chicken *Gallus gallus domesticus*	Air-cell egg injection; mRNA expression in liver	0–10000 ng/g egg	Hepatic tissue: ND –1170 Cerebral cortical tissue: ND –102	D1, D2, D3: – D2: ↑ L-FABP, IGF-1: ↓
Crump et al. (2011) *Comp Biochem Phys C*	Dechlorane Plus	Chicken *Gallus gallus domesticus*	Embryonic hepatocytes and whole embryos	*In vitro:0–3 μM* *In vivo: 0–500 ng/g egg*	Hepatic tissue: ND – 85.6	In vitro and in vivo: D2, D3, TTR, Spot 14, L-FABP, IGF-1: –
Crump et al. (2012) *Toxicol Sci*	TCPP TDCPP	Chicken *Gallus gallus domesticus*	Avian hepatocytes and neuronal cells	2.5 μL/ well 0.01–300 μM	N/A	TDCPP Spot 14, L-FABP, IGF-1: ↓ D1, D2, D3, TTR: – TCPP Spot 14, L-FABP, IGF–-1, TTR: ↓ D1, D2, D3: –
Crump et al. (2014) *Toxicol Appl Pharmacol*	DBE-DBCH TMPP	Chicken *Gallus gallus domesticus*	Air-cell egg injections	DBE-DBCH: 0–54900 ng/g egg TMPP: 0–261400 ng/g egg	DBE-DBCH: Liver: ND-1069 Cerebral: ND-168 Yolk sac: ND-5604 TMPP: ≤0.04–32869 (included ND)	DBE-DBCH (hepatic mRNA expression) D1, D2, D3, IGF1, L-FABP, TTR: – TMPP (hepatic mRNA expression) L-FABP: ↑ TTR: ↓
Crump et al. (2016) *Environ Sci Technol*	TMPP, TBOEP, DBE-DBCH, TCIPP TDCIPP	Double-crested cormorant *Phalacrocorax auritus*	Hepatocytes	0.003–300 μM	N/A	IGF-1 ↓ when exposed to TMPP, DBE-DBCH, SI-BDE-209
Egloff et al. (2011)	HCDBCO, BTBPE,	Chicken	Embryonic hepatocytes	*In vitro*:0.001–30 μM	Hepatic tissue:	HCDBCO: *(continued on next page)*

Table 1b *(continued)*

Paper	Chemical (s)	Species	Tissue	Dose administered	Concentration in tissues (ng/g ww)	Effects
Toxicol Lett	DBDPE, BEHTBP	*Gallus gallus domesticus*	(all four BFRs) Embryos (HCDBCO, BTBPE)	*In vivo*: 0.1–10 μg/g egg	HCDBCO: 0–2428 BTBPE: < 0.15–57	D1, D2, D3, TTR, Spot 14, L-FABP, IGF–1: – BTBPE: D3: ↓ D1, D2, TTR, Spot 14, L-FABP, IGF–1: – DBDPE: D1: ↑ D2, D3, TTR, Spot 14, L-FABP, IGF–1: – BeHTBP: D1, D2, D3, TTR, Spot 14, L-FABP, IGF–1: –
Egloff et al. (2014) *Toxicol Appl Pharmacol*	TBOEP TEP	Chicken *Gallus gallus domesticus*	Air-sac injection; sacrificed at pipping	TBOEP: 0–45400 ng/g egg TEP: 0–241500 ng/g egg	Homogenized embryonic contents: TBOEP: < 0.05–100 (some ND) TEP: ND - < 0.07	TBOEP No effect on hepatic mRNA expression TEP L-FABP: ↑ TTR: ↓ D1, D2, D3, IGF1: –
Farhat et al. (2013) *Toxicol Sci*	TCPP TDCPP	Chicken *Gallus gallus domesticus*	Air cell injection; inc to pipping (d 21–22)	TCPP (ng/g egg): < 0.2–51600 TDCPP (ng/g egg): < 0.06–45000	TCPP; TDCPP: Liver: < 0.2–4.8; < 0.06–2.0 Brain: 0.7–5.8; < 0.06–15 Yolk sac < 0.2–10; 0.9–100	TCPP D1, LFABP significantly induced in liver TDCPP No change in genes of interest
Farhat et al. (2014) *Env Tox Chem*	TDCPP	Chicken *Gallus gallus domesticus*	Embryonic hepatocytes and whole embryos	*In vitro*: 0–10 μM *In vivo*: 0–50 μg/g egg	N/A	Days 12–19 incubation, genes related to steroid hormone metabolism most affected
François et al. (2016) *Env Tox Chem*	PBDEs (31 congeners;congeners not listed)	Ring-billed gull *Larus delawarensis*	Adult	N/A (field study)	Σ31PBDEs in liver: ~128	Liver D1 mRNA expression inversely related to liver Σocta-PBDE levels
Ma et al. (2015) *Environ Toxicol Chem*	TBBPA TBBPA-DBPE	Chicken *Gallus gallus domesticus*	Embryonic hepatocytes	0.01–300 μM	N/A	TBBPA and TBBPA-DBPE: ApoII and VTG: –
Porter et al. (2014) *Environ Toxicol Chem*	16 organic flame retardants[b]	Chicken *Gallus gallus domesticus* Herring gull *Larus argentatus*	Embryonic hepatocyte in vitro screening	0.01–300 μM	N/A	*Thyroid hormone pathway* TEHP, TCP, β- and t-DBE-DBCH, TCPP, TDCPP, TBC altered 1 or more of 4 thyroid-associated genes: D1, IGF-1, NCOA3, THRSP Technical-DBE-DBCH DI1: ↑ IGF1, THRSP: ↓ Most altered gene was IGF1: ↓

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Table 1b *(continued)*

Paper	Chemical (s)	Species	Tissue	Dose administered	Concentration in tissues (ng/g ww)	Effects
						13 and 29 times by TCP and TBC
						Sex steroid pathway
						TEHP, TCP, ATE, BBMP, TDCPP, TBC upregulated VTG2
						Lipid/cholesterol metabolism
						TDCPP ↑ ACSL5, HMGCR and SLCO1A2
						BBMP ↓ genes HMGCR and SLCO1A2
						TCP ↑ genes ACSL5 and SLCO1A2
Técher et al. (2016) *Sci Total Environ*	PBDEs (34 congeners; congeners not listed)	Ring-billed gull *Larus delawarensis*	Adult	N/A (field study)	Σ34PBDEs in liver: ~150	*Thyroid hormone pathway (14 genes)*
						D3, TPO, TRβ in thyroid gland ↓ with liver PBDEs
						In brain, OATP1C1 and TTR ↑ with PBDEs

[a] BDE-17, –28, –47, –66, –71, –85, –99, –100, –138, –153, –154, –183, –190
[b] 16 organic flame retardants: TBPP, TEP, TEHP, TBEP, Mel, CA, HDEHP, VB, TCP, ATE, BBMP, β-DBE-DBCH, t-DBE-DBCH, TCPP, TDCPP, TBC.

Nine studies, including studies on multiple species within the same paper counted as separate studies, examined the thyroid gland, either histology, glandular hormone levels, or the gland's ability to produce T_4 (Egloff et al., 2014; Fernie et al., 2005, 2015; Fernie and Marteinson, 2016; McKernan et al., 2009; Rattner et al., 2013a). Of these, one study examining the effects of four OPEs, including glandular histology (Fernie et al., 2015), two others examining the effects of the commercial PBDE mixture, DE-71, on glandular T_4 production (Fernie and Marteinson, 2016) and glandular mass (Rattner et al., 2013a), all in American kestrels (hatchlings, nestlings, adults), found significant effects (Table 2). However, glandular T_4 content and concentrations were not affected by DE-71 in hatchling American kestrels, chickens, or mallards (*Anas platyrhynchos*) (McKernan et al., 2009), nor in chicken embryos exposed to TBOEP or triethyl phosphate (TEP) (Egloff et al., 2014). Furthermore, unlike the kestrel hatchings, there were no alterations in glandular mass of common tern hatchlings (*Sterna hirundo*) (Rattner et al., 2013a). Together, the results of these studies suggest that the thyroid gland, specifically its structure and ability to produce T_4 (its primary function), may be sensitive measures of FR exposure. In addition, it would appear that changes in the thyroid gland (structure, ability to produce T_4) may be more likely to occur in some avian species than others, and this hypothesis is consistent with the overall findings of species differences in embryotoxicity to PBDEs (McKernan et al., 2009; Rattner et al., 2013a). Additional research characterizing the possible effects of FRs on the avian thyroid gland is warranted.

Several studies examined the effects of FRs on circulating or *in ovo* T_4 and T_3 concentrations. These effects are described below.

Of the 14 studies that examined associations between PBDEs and T_4, four found effects, with two leading to increased T_4 levels (Eng et al., 2014a; Técher et al., 2016) and two to decreased T_4 levels in exposed birds (Fernie et al., 2005; Fernie and Marteinson, 2016) (Table 2). In one study, plasma T_4 concentrations in American kestrel nestlings were negatively correlated with corporal concentrations of the PBDE congeners BDE-47 and BDE-99, but not with sum (∑) PBDEs, and only with BDE-100 at a 0.1 significance level (Fernie et al., 2005). T_4 also decreased with increasing concentrations of HBCD in American kestrels (Marteinson et al., 2011b), and the two OPEs, triethyl phosphate (TEP) (Egloff et al., 2014) and TDCPP (Farhat et al., 2013), in chickens (*Gallus domesticus*) (Table 2). T_4 in plasma was also increased by short-term (7 d) exposure to TBOEP, TCEP and TDCIPP in adult American kestrels (Fernie et al., 2015). No study found an association between T_4 and concentrations of TMPP (Crump et al., 2014), TBOEP (Egloff et al., 2014), and TCIPP (Farhat et al., 2013), studies all conducted with chicken embryos (Table 2). Although there was no correlation between DBE-DBCH and T_4 in two of the captive studies that examined this association, one on chickens (Crump et al., 2014) and the other on American kestrels (Marteinson et al., 2012b), a subsequent study on American kestrels found that circulating total T_4 and free T_4 were lower and higher, respectively, in males exposed to β-DBE-DBCH relative to control males, with no difference between exposed and control females (Marteinson et al. in press) (Table 2).

Of the 13 studies that examined T_3, one study on American kestrels reported a decrease in T_3 in association with PBDE concentrations (Fernie and Marteinson, 2016), and in a study on bald eagles (*Haliaeetus leucocephalus*), an increase in T_3 in association with ∑ hydroxylated (OH-) PBDE (6′-OH-BDE-49, 6-OH-BDE-47, 4-OH-BDE-49) but not ∑PBDE (BDE-47, BDE-100, BDE-99, BDE-153, BDE-138, BDE-183, and BDE-209) (Cesh et al., 2010) (Table 2). In wild peregrine nestlings, neither circulating T_3 nor T_4 concentrations were correlated with ∑PBDEs or ∑OH-PBDE concentrations, despite significant regional differences across the Canadian Great Lakes Basin in T_3 (Smits and Fernie, 2013) and ∑PBDEs (Fernie and Letcher, 2010). The exposure of adult American kestrels to TBOEP, TCEP, TCIPP or TDCIPP, altered their T_3 plasma concentrations overall and

Table 2
Characterizing changes in thyroid parameters of birds exposed to flame retardants.

Paper	Chemical (s)	Species	Exposure method	Dose administered (ng/g bw)	Concentration in tissues (ng/g ww)[a]	Effects
Cesh et al. (2010)*Environ Toxicol Chem*	∑PBDE (BDE−47, −99, 100, −138, −153, −183, −209) ∑OH-PBDE (6′-OH-BDE−49, 6-OH-BDE−47, 4-OH-BDE−49)	Bald eagle *Haliaeetus leucocephalus*	Nestling and adult plasma in the wild	N/A	Field: Nestling ∑PBDE: 0.005−30.61 Nestling ∑OH-PBDE: 0.01−2.1 Nestling HBCD: 0.005 (all samples)	∑PBDE: no correlation with T_3 or T_4 in nestlings and adults ∑OH-PBDE: no correlation with T_4 in nestlings and adults Higher T_3 in individuals with higher ∑OH-PBDE in nestlings only
Crump et al. (2014) *Toxicol Appl Pharm*	DBE-DBCH TMPP	Chicken *Gallus gallus domesticus*	Air-cell egg injections	DBE-DBCH: 0−54900 ng/g egg TMPP: 0−261400 ng/g egg	DBE-DBCH (20−22 d post injection): Liver: ND−1069 Cerebral: ND−168 Yolk sac: ND−5604 TMPP: ≤0.04−32869	Free and bound T_4 levels unaffected by exposure in pipping embryos
Egloff et al. (2014) *Toxicol Appl Pharm*	TBOEP TEP	Chicken *Gallus gallus domesticus*	Air-sac injections	TBOEP: 0−45400 ng/g egg TEP: 0−241500 ng/g egg	Homogenized embryonic contents: TBOEP: < 0.05−100 TEP: ND - < 0.07	Thyroid gland (TT_4[b]): unaltered by TBOEP or TEP in pipping embryos Plasma (FT_4[b]): unaltered by TBOEP; TEP decreased FT_4 in pipping chicks
Eng et al. (2013a) *Environ Pollut*	BDE−99	Zebra finch *Taeniopygia guttata*	Oral exposure for 20 dph[c]	0−173.8 ng/g bw/ d for 20 d	332−14080 ng/g lw (plasma) (Eng et al., 2012 *Tox Sci*)	No effect on concentrations of T_3, FT3, T4, and FT_4 in adults
Eng et al. (2014a) *Ecotoxicology*	BDE−99	European starling *Sturnus vulgaris*	Oral exposure for 20 dph	0, 15.8, 173.8 ng/g bw/d for 20 d	332−14080 ng/g lw (plasma) (Eng et al., 2012 *Tox Sci*)	Exposed fledglings higher FT_4 No effect on fledgling FT_3, T_3, T_4 No effect on adult FT_4, T_4, FT_3, T_3
Eng et al. (2014b) *Sci Total Environ*	∑PBDE[d]	European starling *Sturnus vulgaris*	Eggs at agricultural site; condition of mothers in relation to her eggs in wild	N/A	Field: ∑PBDE in eggs Geometric mean: 10.9 Range: 2−307	∑PBDE in eggs not correlated with T_4 concentration in mothers or chicks from same nest
Farhat et al. (2013) *Toxicol Sci*	TCPP TDCPP	Chicken *Gallus gallus domesticus*	Injection in air cell before incubation; incubated until pipping (day 21−22)	TCPP (ng/g egg): < 0.2−51600 TDCPP (ng/g egg): < 0.06−45000	TCPP (ng/g ww): Liver < 0.2−4.8 Cerebral hemisphere 0.7−5.8 Yolk sac < 0.2−10	TDCPP reduced plasma FT_4 at one concentration in embryos
Fernie et al. (2005) *Toxicol Sci*	∑PBDE (BDE−47, −99, −100, −153)	American kestrel *Falco sparverius*	*In ovo* injection and 29 dph exposure by diet	*In ovo*: 1430 ng/g egg Nestling: 15.6 ng/g bird /d for 29 d	Whole chick homogenization at 36 dph: 0.72−86.09 (BDE−47, −99, −100, −138, −153, −183)	Plasma TT_4 concentration in nestlings negatively correlated with BDE−47 and BDE−99 (not ∑PBDE) No effect on TT3 plasma concentration in nestlings No effect on morphology and pathology of thyroid gland in nestlings

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Table 2 *(continued)*

Paper	Chemical (s)	Species	Exposure method	Dose administered (ng/g bw)	Concentration in tissues (ng/g ww)[a]	Effects
Fernie et al. (2015) *Environ Sci Technol*	OPEs: TBOEP, TCEP, TCIPP, TDCIPP	American kestrel *Falco sparverius*	Adult exposure via diet	22 ng/g kestrel/d for 21 d	Immediately after exposure: Liver: less than ≤0.1 for all except TBOEP: 4–5 Kidney: less than ≤0.1	Overall effects of chemicals: increases in FT_3 (32–96%) in adults One or more chemical increase TT_3, TT_4, FT_3, and FT_4 in adults Effects on thyroid gland and T_4-ORD (conversion of T_4- T_3) activity in adults
Fernie and Marteinson (2016) *Environ Toxicol Chem*	DE–71 (BDE–28, –47, –100, –99, –154, –153, –183)	American kestrel *Falco sparverius*	Nestlings exposed *in ovo* by maternal transfer	Mothers: 0–320 ng/g bw/d for 75 d	∑PBDE[e] in ovo; will grow to be subjects; tested at 17–20 dph 0–1131	<u>Female nestlings:</u> Reduced TT_4 released by thyroid gland after TSH challenge; baseline TT_3 lower in dosed group Circulating plasma TT_3 and TT_4 significantly influenced overall Modified response of thyroid gland to produce TT4 after TSH challenge <u>Male nestlings:</u> No effect of exposure
Marteinson et al. (2011a) *Toxicol Sci*	DE–71 (BDE–28, –47, –100, –99, –154, –153, –183)	American kestrel *Falco sparverius*	Embryonic exposure via maternal transfer	Mothers: 0–320 ng/g bw /d for 75 d	In sibling eggs (DE–71[f]): 3–1131 In sibling eggs (HBCD unexpectedly): 0.002–16	No effect on concentrations of FT_3 or FT_4 during breeding in adults
Marteinson et al. (2011b) *Environ Res*	HBCD	American kestrel *Falco sparverius*	Adult males exposed via diet	Unpaired: 0–510 ng/g bw/ d for 21 d Paired: 0–510 ng/g bw/ d for 75 d	Unpaired plasma at end of uptake: 0.26–18.50 Unpaired plasma at end of 21 d depuration: 0.09–2.83 Paired in their eggs: 0.6–179.9	TT_4 and FT_4 lower in plasma in adult males exposed in ovo than in control males
Marteinson et al. (2012b) *Environ Sci Technol*	DBE-DBCH	American kestrel *Falco sparverius*	Breeding pairs diet for 82 d Maternal transfer to eggs examined	0–0.239 ng/g bw/ d for 82 d	After 4-weeks: subset of individuals sacrificed No DBE-DBCH detected in liver, fat, plasma, and egg samples	No effect on T_3 and T_4 concentrations in eggs
Marteinson et al. (In press)	DBE-DBCH	American kestrel *Falco sparverius*	Adult pairs orally exposed	0–0.239 ng/g bw/ d for 82 d	DBE-DBCH and metabolites not detected in tissues or eggs laid by pairs	Exposed males ↓ TT4 but ↑ FT4 than controls No effect on females
McKernan et al. (2009) *Environ Toxicol Chem*	DE–71 (BDE–17, –28, –47, –49, –66, –85, –99, –100, –153, –154)	Mallard *Anas platyrhynchos* Chicken *Gallus gallus domesticus* American kestrel *Falco sparverius*	Air-cell egg administration; euthanized 1 dph	0–20000 ng/g egg	Chicken: ND–4930 Kestrel: 0.38–2800	No affect for three species on glandular T_4 in eggs
Nost et al. (2012)	∑PBDE[g]	Northern fulmar	Chick plasma in the wild	N/A	Field:	No correlation between FT_3, TT_3, FT_4, TT_4

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Table 2 *(continued)*

Paper	Chemical (s)	Species	Exposure method	Dose administered (ng/g bw)	Concentration in tissues (ng/g ww)[a]	Effects
Sci Total Environ		*Fulmarus glacialis* Black-legged kittiwake *Rissa tridactyla*			Black-legged kittiwake: 0.09 Northern fulmar: 0.17	and ∑PBDEs in chicks
Rattner et al. (2013a) *Chemosphere*	DE–71 (congeners not listed)	American kestrel *Falco sparverius* Common tern *Sterna hirundo*	Air-sac egg injection; chicks euthanized at hatching	Tern and kestrel: 0–20000 ng/g egg	Control eggs (infertile or dead early incubation): Tern: < 52 Kestrel: < 4	No effect on organ-to-body weight ratio of thyroid in eggs Thyroid histology: uniform among DE–71 treated and control eggs
Smits and Fernie (2013) *Comp Immunol Microb*	∑PBDE (14 congeners; not listed) ∑OH-PBDE (not listed)	Peregrine falcon *Falco peregrinus*	Chick plasma in the wild	N/A	Field: Not reported	No significant correlation between T_3/ T_4 and ∑PBDE/∑OH-PBDE in chicks
Técher et al. (2016) *Sci Total Environ*	PBDEs (34 congeners; not listed)	Ring-billed gull *Larus delawarensis*	Adult plasma in the wild	N/A	Σ_{34}PBDEs in liver: ~150	Positive correlation between hepatic PBDEs and plasma T_4
Van den Steen et al. (2010) *Sci Total Environ*	∑PBDE[h]	European starling *Sturnus vulgaris*	Exposed over 6 months via subcutaneous implants	0–1740 ng/g bw	Plasma: 0.151–23400 ng/ml (=ng/g ww)	No effect on T_3 and T4 in adults
Verrault et al. (2007) *Environ Pollut*	∑PBDE[i] ∑OH-PBDE[j] ∑MeO-PBDE[k]	Glaucous-winged gull *Larus glaucescens*	Plasma in breeding adults in the wild	N/A	Field: ∑PBDE: females 19.3; males 21.3 ∑MeO-PBDE: females 0.67; males 1.00 ∑OH-PBDE: females 0.33; males 0.44	FT_3, TT_3, FT_4, TT_4 not associated with BMR or any FR in adults
Winter et al. (2013) *Environ Toxicol Chem*	BDE–99	Zebra finch *Taeniopygia guttata*	Egg injection; bred for three generations	0–1000 ng/g egg	Not reported	No effect on FT_3, TT_3, TT_4, FT_4 in adults

[a] The congeners in tissues are the same as those in the Chemicals column, unless otherwise noted.
[b] FT_4 = Free T_4 / TT_4 = Total T_4.
[c] dph=days post hatch.
[d] 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, 209.
[e] BDE-17, −28, −47, −49, −66, −85, −99, −100, −138, −153, −154, −183, −190, −209.
[f] BDE-17, −28, −47, −49, −66, −85, −99, −100, −138, −153, −154, −183, −190, −209.
[g] BDE-28, −47, −71/49, −66, −77, −99, −100, −85, −119, −153, −154, −183, −196, −206, −209.
[h] BDE-28, −47, −49, −66, −85, −99, −100, −153, −154, −183.
[i] BDE-28, −47, −66, −85, −99, −100, −138, −153, −154 (co-elution with brominated biphenyl 153), −183.
[j] 4-OH-BDE42, 6-OH-BDE47, 3-OH-BDE47, 4′-OH-BDE49, 6′-OH-BDE49, 2′-OH-BDE68.
[k] 2′-MeO-BDE28, 4-MeO-BDE42, 6-MeO-BDE47, 3-MeO-BDE47, 4′-MeO-BDE49, 6-MeO-BDE90/6-MeO-BDE99.

increased T_3 at 7 d exposure (Fernie et al., 2015). Taken together with our results from the thyroid gland, we argue that, as well as PBDEs and their metabolites, and HBCD, some novel FRs, including OPEs, may be implicated in altered thyroid status and deserve further study in research simultaneously assessing the complete suite of thyroid endpoints (e.g., glandular changes, circulating thyroid hormone levels).

Effects on the thyroid parameters seemed to differ between the ages of the subjects. Considering only studies that involve PBDEs, and considering each species within papers that included multiple species to be separate studies, none of the five laboratory studies (American kestrel, chicken, and mallard within McKernan et al. (2009) and American kestrel and common tern within Rattner et al. (2013b)) that determined T_3 or T_4 levels in the egg showed an association with any of the measured PBDE congeners (Table 2). Similarly, none of the seven studies (two field studies and five laboratory studies) that examined adults (defined here as post-fledging) found an effect on T_3 and T_4 concentrations (Cesh et al., 2010; Eng et al., 2014a, 2013a, 2013b; Marteinson et al., 2011b; Van den Steen et al., 2010; Verreault et al., 2007; Winter et al., 2013) (Table 2). However, out of six studies that examined nestlings (Cesh et al., 2010; Eng et al., 2014a; Fernie et al., 2005; Marteinson and Fernie 2016; Nøst et al., 2012 [2 species]), including three field studies, four found an effect. Specifically, Cesh et al. (2010) reported higher T_3 in individuals with higher ∑OH-PBDE in nestling bald eagles, but not in adults. Likewise, European starling (Eng et al., 2014a) and American kestrel (Fernie et al., 2005) nestlings exposed to PBDEs had higher T_4 concentrations. Finally, female American kestrel nestlings exposed to the commercial PBDE mixture, DE-71 (e.g., BDE-47, −99, −100, −153, −153, and −154), experienced reduced T_3 and an overall change in T_3 and T_4, in addition to reduced T_4 released by the thyroid gland after a TSH challenge (Fernie and Marteinson, 2016). In a meta-analysis based primarily on organochlorines by Cesh et al. (2010), thyroid status was impacted by contaminants at the nestling, but not adult stage. Perhaps nestling birds are less able to establish and maintain thyroid homeostasis than adult birds due to the simultaneous demands relating to growth and thermoregulation. We argue that future studies should focus on the nestling stage for laboratory studies and involve exposure to a FR at the embryonic and/or nestling stages as occurs with wild birds.

Disruption of the thyroid system can have far-reaching effects at different hierarchical levels within the AOP. Because the thyroid system plays an important role in growth, thermoregulation and metabolism, its disruption can lead to abnormal changes in these parameters, which in turn can affect the survival of chicks and adults, endpoints described below. Disruption of other hormones, such as steroids, can also lead to changes at the level of the whole organism.

3.3. Steroids

Three main steroids have been the subject of research characterizing the effects of only a limited number of flame retardants in birds; corticosterone and the sex steroids, testosterone and estradiol.

Corticosterone, the main glucocorticoid in birds and the end product of the hypothalamic-pituitary-adrenal (HPA) axis, is elevated in the plasma of birds undergoing physiological or psychological stress (Astheimer et al., 1992). In response to a stressful stimulus, the hypothalamus releases corticotropin releasing hormone, stimulating the pituitary to release adrenocorticotropin hormone, which then stimulates the adrenal glands to release corticosterone. High levels of corticosterone in the plasma will stimulate the liver to convert glycogen to glucose, which facilitates the response to stress (e.g., fight or flight). Finally, corticosterone exhorts negative feedback on the hypothalamus to establish homeostasis. Corticosterone plays a critical role in initiating food searching behaviour in adult birds, and begging and aggression in chicks (Astheimer et al., 1992; Kitaysky et al., 2003). Elevated corticosterone may also reduce cognitive functioning in birds, although it may enhance cognitive functioning under different exposure and testing methods (Bebus et al., 2016; Kitaysky et al., 2003).

In the current review, there was no clear pattern of effect of FRs on corticosterone (Table 3). This lack of pattern may be related to the limited number of studies available for assessment and/or the different matrices in which corticosterone was measured (blood, eggs, feathers) between studies, making comparisons difficult. Specifically, corticosterone in blood reflects current stress status, which can change rapidly (i.e., 3 min) in response to a stressor (Romero and Remage-Healey, 2000), whereas feather corticosterone is a long-term, integrated measure of stress to the individual (Bortolotti et al., 2008) and corticosterone in eggs is influenced by maternal stress (Pitk et al., 2012). The studies included in our review involved assessments of corticosterone relative to birds being exposed to PBDEs in the field (Bourgeon et al., 2012; Verboven et al., 2010), or to the replacement BFR, DBE-DBCH, in the laboratory (Marteinson et al., 2012b). Of three studies, two reported effects on corticosterone, with opposite results. In the two field studies, free-living great skuas (*Catharacta skua*) had decreased feather corticosterone concentrations in relation to their exposure to PBDEs (Bourgeon et al., 2012), whereas glaucous gulls had increased baseline plasma corticosterone but decreased stress-induced plasma corticosterone in relation to PBDE concentrations (Verboven et al., 2010) (Table 3). In the single lab exposure study, American kestrels exposed to β-DBE-DBCH experienced no change in corticosterone levels in their eggs (Marteinson et al., 2012b) (Table 3). The effects of FRs on other steroids (i.e., sex steroids) were more widely reported in the literature.

The sex steroids, testosterone (T) and estradiol (E_2), are the end products of the hypothalamic-pituitary-gonadal axis (HPG) (Ottinger et al., 2002). The hypothalamus produces gonadotropin-releasing hormone, which stimulates the pituitary to release luteinizing hormone and follicle-stimulating hormone, in turn stimulating the gonads to produce testosterone and estradiol. Birds exposed to stress early in life can have an altered HPG axis as adults. For example, adult song sparrows (*Melospiza melodia*) experiencing elevated levels of corticosterone as nestlings exhibited lower levels of estradiol and higher levels of testosterone, respectively (Schmidt et al., 2014). An altered HPG axis may also negatively affect adult sexual behaviour and reproduction in birds (Nowicki et al., 2002; Schmidt et al., 2013; Spencer et al., 2003) and in mammals (Guzmán et al., 2006; Rhind et al., 2001). Associated with these behavioural differences, sexual differentiation in the brain may also be altered because sex steroids play a critical role in the development of brain structure and function that result in sexual dimorphism (Ottinger et al., 2002).

Several studies examined levels of T and E_2 in the context of FR exposure. Out of six studies which examined possible associations between FRs and T (Marteinson et al., 2011b, 2011a, 2012b; Marteinson et al. in press; Van den Steen et al., 2010; Verreault et al., 2006), four studies examined PBDEs and their metabolites, two studies examined HBCD (one studied both HBCD and PBDEs), and two studies examined β-DBE-DBCH (Table 4). One of the four studies that examined PBDEs found significant associations, with positive associations between concentrations of T and PBDEs, and between T and the ∑MeO-PBDE concentrations in eggs of glaucous gulls (Verboven et al., 2008) (Table 4). One of the two studies that examined HBCD found a significant relationship between the HBCD concentrations measured in eggs and the plasma T of paired American kestrel males but not of unpaired males, with T in HBCD-exposed paired males being higher when the first egg was laid than in control paired males (Marteinson et al., 2011b) (Table 4). The other study that examined HBCD reported a positive association between HBCD and T measured in the yolk of unincubated glaucous gull eggs, and also found that the relative ratio of T and E_2 in the eggs was altered in association with the gulls' varying exposure to HBCD (Verboven et al., 2008). T concentrations in the first laid egg of American kestrels pairs was unrelated to the exposure of these pairs to β-DBE-DBCH (Marteinson et al., 2012b). In the same adult kestrels exposed to β-DBE-DBCH, circulating T was higher in

Table 3
Circulating corticosterone changes in birds in the context of flame retardant exposure.

Paper	Chemical (s)	Species	Exposure method	Dose administered (ng/g bw)	Chemical concentration in tissues (ng/g ww)[a]	Effect
Bourgeon et al. (2012) *Environ Res*	∑PBDE[b]	Great skua *Catharacta skua*	Plasma of adults in the wild	N/A	Field (from different sites): 7.06–32.28	Increased plasma PBDEs correlated with decreased feather corticosterone
Marteinson et al. (2012b) *Environ Sci Technol*	β-DBE-DBCH	American kestrel *Falco sparverius*	Breeding pairs exposed by diet	0–0.239 ng/g bw/ d for 82 d	After 4-weeks: subset of individuals sacrificed No DBE-DBCH in liver, fat, plasma, and egg	No effect on corticosteroid levels
Verboven et al. (2010) *Gen Comp Endocr*	∑PBDE[c] ∑Meo-PBDE[d]	Glaucous gulls *Larus hyperboreus*	Plasma in incubating adults in wild	N/A	Field: ∑PBDE: Females: 8.70, Males: 16.30 ∑OH-PBDE: Females: 0.94, Males: 1.79	Positive correlation between baseline plasma cort and POP (including PBDE) Negative correlation between stress-induced plasma cort and POP (including PBDE)

[a] The congeners in tissues are the same as those in the Chemicals column, unless otherwise noted.
[b] BDE-47, −66, −100, −119, −99, −154, −153, −183.
[c] BDE-17, −25, −28, −47, −49, −54, −66, −75, −77, −85, −99, −100, −116, −119, −138, −139, −140, −153, −154/BB153, −155, −156, −171, −180, −181, −183, −184, −190, −191, −196, −197, −201, −202, −203, −205, −206, −207, −208, −209.
[d] 6′-OH-BDE17, 4′-OH-BDE17, 2′-OH-BDE28, 4-OH-BDE42, 5-OH-BDE47, 6-OH-BDE47, 3-OH-BDE47, 4′-OH-BDE49, 6′-OH-BDE49, 2′-OH-BDE68, 6-OH-BDE85, 6-OH-BDE90, 6-OH-BDE99, 2-OH-BDE123 and 6-OH-BDE137.

exposed males but lower in treatment females, and despite the changes in circulating E_2 in the same females (discussed below), their circulating T:E_2 ratio remained unchanged despite their exposure to β-DBE-DBCH (Marteinson et al. in press).

Only four studies have examined sex steroids other than T (Marteinson et al., 2012b, Marteinson et al. in press, Van den Steen et al., 2010, Verboven et al., 2008, Verreault et al., 2006) (Table 4). American kestrel pairs exposed to β-DBE-DBCH by diet had higher concentrations of E_2 and estrone in their first laid egg than unexposed pairs (Marteinson et al., 2012b), while maternal circulating E_2 concentrations were lower in the exposed females prior to egg laying and were not correlated with the estrogens measured in their eggs; E_2 was undetected in males (Marteinson et al. in press). While one study found no association between E_2 and PBDE in the plasma of adult European starlings (Van den Steen et al., 2010), a second study found a positive association between PBDEs and E_2, but not between HBCD and E_2 in eggs of glaucous gulls (Verboven et al., 2008). Finally, the only study examining progesterone found a positive association between this steroid measured in plasma and plasma concentrations of PBDEs in male, but not in female glaucous gulls (Verreault et al., 2006).

Although based on small sample sizes, our overall synthesis, with multiple FRs within a paper considered as separate studies, indicates that corticosterone (67% of studies found an effect; n =3) and progesterone (100%; n =1) need further assessment in future research since these two hormones may be more susceptible to possible effects in birds that are exposed to FRs, than T (43%; n=7) and E_2 (50%; n=4). Given that we reported a single study on progesterone, we urge future investigators to study several steroids simultaneously and appropriately depending on the endocrine mode of action of the FRs (e.g., DBE-DBCH is an androgen-agonist depending on the isomer, while most other FRs will not be). Recent advances in liquid chromatography–mass spectrometry (LC-MS) technology means that it is now possible to measure several steroids simultaneously in a single sample. Such an approach is promising as one of the studies found a relationship with the ratio of T:E_2 and the measured FRs, but no relationships with either of the two individual hormones, T or E_2 (Verboven et al., 2008). However, the variations in circulating T, E_2 and T:E_2 that occur in relation to the avian reproductive cycle, may confound measurements of potential relationships with FRs. Thus, in combination with addressing these cyclical endocrine changes, the relative relationship of these two hormones (or of all four hormones; T, E_2, progesterone and corticosterone), concurrent with the absolute concentrations of each hormone, could be a useful future direction of study.

Disruption in one or multiple of these steroid hormones can lead to several other changes within the avian AOP. With changes in steroid hormones, reproductive behaviours of adults and begging behaviour of chicks may be altered, suggesting one possible mechanism for some of the observed behavioural changes in birds exposed to FRs, and even possibly changes in the growth and development of nestlings.

3.4. Retinol

Retinol (vitamin A_1) is the most commonly measured vitamin in the context of flame retardant research in birds. It plays a critical role in growth, development, and reproduction (Fraser and Bramley, 2004; Tanumihardjo, 2011). Carotenoids, another component of vitamin A, play an especially important role in birds because they influence plumage colour (Fraser and Bramley, 2004), an important trait in sexual selection, and vision (Tanumihardjo, 2011), which is a key sense for most birds, especially visual predators like raptors. The liver is the main storage organ of retinol and therefore measures of liver reserves are considered the “gold standard” in monitoring the health of individuals and populations (Tanumihardjo, 2011).

Out of eight studies that examined associations between retinol and PBDEs and OH-PBDE metabolites (Cesh et al., 2010; Fernie et al., 2005; Miljeteig et al., 2012; Murvoll et al., 2005, 2006a, 2006b; Smits

Table 4
Changes in sex steroid hormone concentrations in birds exposed to FRs.

Paper	Chemical (s)	Species	Exposure method	Dose administered (ng/g bw)	Chemical concentration in tissues (ng/g ww)[a]	Effect
Marteinson et al. (2011b) *Environ Res*	HBCD	American Kestrels *Falco sparverius*	Adult males exposed via diet	Unpaired: 0–510 ng/ g bw/ d for 21 d Paired: 0–510 ng/g bw/ d for 75 d	Unpaired plasma at end of uptake period: 0.26–18.50 Unpaired plasma at end of 21 d depuration: 0.09–2.83 Paired in their eggs: 0.6–179.9	Unpaired exposed males did not have higher T than controls Exposed breeding males had higher T when first egg was laid and T followed a different temporal pattern in exposed males than in controls
Marteinson et al. (2011a) *Toxicol Sci*	DE–71[b]	American Kestrels *Falco sparverius*	Embryonic exposure via maternal transfer	Mothers: 0–320 ng/g bw /d for 75 d	In sibling eggs (DE–71[c]): 3–1131 In sibling eggs (HBCD unexpectedly): 0.002–16	T did not vary over time and was not associated with *in ovo* concentrations of PBDEs
Marteinson et al. (2012b) *Environ Sci Technol*	β-DBE-DBCH	American Kestrel *Falco sparverius*	Breeding pairs exposed by diet for 82 d	0–0.239 ng/g bw/ d for 82 d	After 4-weeks: subset of individuals sacrificed No β-TBECH detected in liver, fat, plasma, and egg but	Estradiols was higher in the first laid-egg of exposed pairs, no difference in T
Marteinson et al. In press	DBE-DBCH	American kestrel *Falco sparverius*	Adult pairs orally exposed	0–0.239 ng/g bw/ d for 82 d	DBE-DBCH and metabolites not detected in tissues or eggs laid by pairs	Exposed males ↑ T than controls; E_2 not detectable Exposed females ↓ T and ↓ E2 than controls
Van den Steen et al. (2010) *Sci Total Environ*	∑PBDE[d]	European Starlings *Sturnus vulgaris*	Exposed over 6 months via subcutaneous implants	0–1740 ng/g bw	Plasma: 0.151–23400 ng/ml (=ng/g ww)	No effect on T and E_2 in plasma of adults
Verboven et al. (2008) *Comp Biochem Phys C*	∑PBDE[e] ∑MeO-PBDE[f] α-HBCD	Glaucous Gull *Larus hyperboreus*	Yolk of unincubated third laid egg in the wild	N/A	Field: α-HBCD: 13–23 ∑PBDE: 131.6–184.7 ∑MeO-PBDE: 15.5–24.6	Positive correlation with ∑MeO-PBDE and yolk T at one of three sites Positive relationship of T and E_2 with PBDE but only T with HBCD Contaminant levels (∑PBDE and ∑-α-HBCD) changed based on the relative concentrations of T and E_2
Verreault et al. (2006) *Environ Toxicol Chem*	∑PBDE[g]	Glaucous Gull *Larus hyperboreus*	Plasma of incubating female and males in the wild	N/A	Field: Females: 14.4 Males: 20.0	Progesterone positively associated with ∑PBDE in males, but not in females No association with T for both sexes

[a] The congeners in tissues are the same as those in the Chemicals column, unless otherwise noted.
[b] BDE-28, −47, −100, −99, −154, −153, −183.
[c] BDE-17, −28, −47, −49, −66, −85, −99, −100, −138, −153, −154, −183, −190, −209.
[d] BDE-28, −47, −49, −66, −85, −99, −100, −153, −154, −183.
[e] BDE-28, −49, −47, −100, −119, −99, −116, −155, −154/153, −153, −138.
[f] 6′-MeO-BDE17, 6-MeO-BDE47, 3-MeO-BDE47, 4-MeO-BDE42.
[g] BDE-47, −99. −100, −153, −154 (coelution with brominated biphenyl 153), −183.

and Fernie, 2013; Sullivan et al., 2010), four reported significant associations (Table 5). One study found a positive association with the sum of OH-PBDE metabolites in bald eagles (Cesh et al., 2010), while the other three studies reported negative associations with the PBDE congener, BDE-100, in American kestrels (Fernie et al., 2005), as well as BDE-100, −153, and ∑PBDEs when kestrels were exposed to DE-71, a PBDE technical mixture (Sullivan et al., 2010), and with ∑PBDEs but not with ∑OH-PBDEs in peregrine falcons (Smits and Fernie, 2013) (Table 5). The other four studies, including three field studies on black-legged kittiwakes (*Rissa tridactyla*), European shags (*Phalacrocorax aristotelis*), and ivory gulls (*Pagophila eburnean*) (Miljeteig et al., 2012; Murvoll et al., 2006a, 2006b), and a mallard laboratory study (Murvoll et al., 2005), did not find an association with retinol and FR exposure (Table 5). Our conclusion is that retinol is moderately sensitive to FRs compared to some of the other endpoints examined in this review, although it may be most sensitive in raptor species.

Disruption in retinol levels can produce several subsequent changes within the AOP. Because retinol and other components of vitamin A play critical roles in growth, development, reproduction, vision, and sexual selection, disruption in retinol levels can affect an individual's survival and reproduction, leading to population-level effects. For example, maternal and nestling retinol levels were correlated with hatching success and growth, respectively, in American kestrels exposed to DE-71 (Sullivan et al., 2010).

3.5. Brain

Toxicological studies examining effects of chemicals on the avian brain have primarily focused on the song control system (SCS) of songbirds. The SCS is comprised of interconnected brain nuclei that control song production and learning (Nottebohm, 2005). Commonly-measured nuclei are HVC (formerly referred to as the high vocal center), robust nucleus of the arcopallium (RA), and Area X. The SCS shows remarkable seasonal plasticity (Nottebohm, 1981) and some of the largest neuroanatomical sex differences seen in any vertebrate (Nottebohm and Arnold, 1976). These sex and seasonal differences are mediated in part by sex steroids during development and during adulthood, respectively (Tramontin and Brenowitz, 2000; Nottebohm et al., 1986). Indeed, androgen and estrogen receptors are expressed in the SCS and other forebrain regions of songbirds (Gahr et al., 1993; Soma et al., 1999). Changes in the SCS are closely linked to changes in sexual behaviour, namely the rate of singing and singing stereotypy (i.e., the consistent repetition of a song) (Tramontin and Brenowitz, 2000).

Of the two studies that examined the brain, all in relation to the PBDE congener, BDE-99, and in songbirds in the laboratory, one study on European starlings found no effect on the volumes of HVC, RA, and Area X (Eng et al., 2014a), and one study on zebra finches (*Taeniopygia guttata*) found that HVC was smaller in exposed females, but no difference was detected in the HVC, RA, and Area X of males and in RA of females (Eng et al., 2012) (Table 6). It appears that the brain of some songbirds may be impacted by BDE-99, but that the associations are possibly sex specific. Also, birds in the two published studies were not exposed during embryonic development, which could have had a more pronounced effect on brain development. There is critical need to examine the effects of FRs on more fine-scale measures of the avian brain. In particular, future studies should continue with broad-scale volumetric measures of different brain regions (i.e., neuroanatomy), like the two studies in our review, in conjunction with more fine-scale measurements such as immediate early gene expression and neurogenesis that are closely linked to brain activity and plasticity. Finally, exposure to FRs during embryogenesis and its subsequent effects on the brain should be included in future studies. Changes in the brain as a result of exposure to FRs may directly affect behaviours related to reproduction, such as song in songbird species, when the song control nuclei are affected, but may also potentially alter other behaviours such as predatory success or predation-avoidance if other parts of the brain are affected. As such, changes in the brain could produce population-level effects via behaviour within the avian AOP.

3.6. Behaviour

Song, vocalizations, and non-vocal interactions between members of a pair are the primary behavioural measurements collected in the context of flame retardant exposure studies in birds. These behaviours are intrinsically connected with reproduction. For example, songbird males involved in extra-pair copulations with larger song-repertoire sizes, have more offspring and their offspring are more likely to survive, than males with smaller song-repertoire sizes (Hasselquist et al., 1996). Birds that do not sing, such as raptors, will emit vocalizations indicative of “friendly” approaches between members of the pair (Smallwood and Bird, 2002), and these are important for establishing the pair-bond and determining the quality of the pair-bond. Non-vocal interactions between members of a pair, such as courtship displays, may also affect reproduction. For example, male American kestrels that spend more time with their mate copulate more frequently (Smallwood and Bird, 2002). Another behavioural endpoint closely related to reproduction is nest or incubation temperature; the more time adults spend incubating their clutch, the higher the nest temperature. In sum, changes in several types of behaviour can have downstream effects, especially on reproductive success, and thus potentially generate population-level changes.

Of the seven studies that examined associations between FRs and behaviour (Eng et al., 2012; Fernie et al., 2008; Marteinson et al., 2010, 2012a, 2015; Sullivan et al., 2013; Verboven et al., 2009), all found statistically significant effects, although not always with the same behaviour and in the same direction (Table 7). Five studies examined the effects of PBDEs on behaviour in zebra finches in the laboratory (Eng et al., 2012), American kestrels in the laboratory (Fernie et al., 2008; Marteinson et al., 2010; Sullivan et al., 2013), and glaucous gulls in the field (Verboven et al., 2009). The behaviours examined were primarily related to reproduction, although this may reflect the focus of the studies in which other behaviours may not have been examined. For example, in PBDE exposure studies, exposed male zebra finches sang less (Eng et al., 2012), exposed American kestrels copulated less frequently (Fernie et al., 2008; Marteinson et al., 2010) and had longer incubation periods with lower nest temperatures (Sullivan et al., 2013), and glaucous gulls exposed to higher PBDE levels also had lower nest temperatures (Verboven et al., 2009) (Table 7). Although significant at the α=0.1 significance level, American kestrels exposed to PBDEs also spent less time in their nest box and males spent less time engaging in pair-bonding behaviours (Fernie et al., 2008). The only study on HBCD found similar impacts, including reduced vocalizations and pair bonding (Marteinson et al., 2012a) (Table 7). In that study, male American kestrels had reduced parental behaviour but females were able to compensate by increasing their parental behaviour. However, nests of the HBCD-exposed birds had lower incubation temperatures, suggesting that females spent less time on the eggs because incubation is mostly carried out by females (Marteinson et al., 2012a). Finally, exposure to β-DBE-DBCH increased androgen-dependent behaviours and reduced courtship and copulation frequency in American kestrels (Marteinson et al., 2015) (Table 7). Clearly, compared to some of the other physiological and biochemical endpoints reviewed here, the behaviour of birds appears to be strongly affected by their exposure to FRs. Because we showed above that the brain may also be impacted by FRs (1 of 2 studies finding an effect), especially if fine-scale measurements of the brain are assessed in future research, we argue that examining the brain concurrently with behaviour would be a fruitful avenue of study.

With changes in behaviour, growth, development, reproduction and survival of birds may be altered, providing one mechanism by which exposure to FRs affects a bird's fitness, as well as avian populations,

Table 5
Assessments of retinol (plasma, hepatic concentrations) in birds in relation to various FRs.

Paper	Chemical (s)	Species	Exposure method	Dose administered (ng/ g bw)	Tissue concentration (ng/g ww)[a]	Effect
Cesh et al. (2010) *Environ Toxicol Chem*	∑PBDE[c] ∑OH-PBDE[d]	Bald eagle *Haliaeetus leucocephalus*	Nestling plasma (wild)	N/A	Field: Nestling ∑PBDE: 0.005–30.61 Nestling ∑OH-PBDE: 0.01–2.1 Nestling HBCD: 0.005 (all samples)	Plasma retinol: 1) Independent of ∑PBDE 2) Positively correlated with ∑OH-PBDE
Fernie et al. (2005) *Toxicol Sci*	∑PBDE (BDE–47, –99, –100, –153)	American kestrel *Falco sparverius*	Egg injection + 29 dph oral exposure	In ovo: 1430 ng/g egg Nestling: 15.6 ng/g bw/d for 29 d	Whole chick homogenization 36dph[b]: 0.72–86.09 (BDE–47, –99, –100, –138, –153, –183)	BDE–100 negative association with plasma retinol No effect on retinol and retinyl palmitate in liver and plasma
Miljeteig et al. (2012) *Sci Total Environ*	∑HBCD (α, β, γ) ∑PBDE (BDE–28, –47, –99, –100, –153, –154)	Ivory gull *Pagophila eburnea*	Eggs (wild)	N/A	Not reported	No correlation with retinol concentrations
Murvoll et al. (2005) *J Toxicol Env Health A*	BDE–99	Mallard *Anas platyrhynchos*	Egg injection; Hatched chicks immediately euthanized	0–10	Yolk sac: 0.2–19.9	No effect on retinol and retinyl palmitate in liver and plasma
Murvoll et al. (2006a) *Environ Toxicol Chem*	∑PBDE[e] HBCD	Black-legged kittiwakes *Rissa tridactyla*	Yolk sac of kittiwake hatchlings (wild)	N/A	Field: ∑PBDE: 419–653 ng/g lw HBCD: 111–335 ng/g lw	No correlation with retinol in liver and plasma
Murvoll et al. (2006b) *Environ Toxicol Chem*	∑PBDE[e] HBCD	European shag *Phalacrocorax aristotelis*	Yolk sac of shag hatchlings (wild)	N/A	Field: ∑PBDE: 17.2 ng/g ww OR 251 ng/g lw HBCD: 28.5 ng/g ww OR 417 ng/g lw	No correlation with retinol in liver and plasma
Smits and Fernie (2013) *Comp Immunol Microb*	∑PBDE (14 congeners; not listed) ∑OH-PBDE (not listed)	Peregrine falcon *Falco peregrinus*	Chick plasma in the wild	N/A	Field: Not reported	Negative correlation with retinol for ∑PBDE, but not with ∑OH-PBDE
Sullivan et al. (2010) *J Toxicol Env Health A*	DE–71[f]	American kestrels *Falco sparverius*	Adults exposed by diet; Nestlings exposed via maternal transfer	0–352 ng/g bw/d for 75 d	First egg of each pair: ∑PBDE total: 3 –1131 (congeners not listed)	Negative correlation with plasma retinol

[a] The congeners in tissues are the same as those in the Chemicals column, unless otherwise noted.
[b] dph=days post hatch.
[c] BDE-47, –99, 100, –138, –153, –183, –209.
[d] 6′-OH-BDE-49, 6-OH-BDE-47, 4-OH-BDE-49.
[e] BDE-28, –47, –99, –100, –153, –154.
[f] BDE-17, –28, –47, –49, –66, –85, –99, –100, –138, –153, –154, –183, –209.

Table 6
Changes in the avian brain with exposure to various flame retardants.

Paper	Chemical (s)	Species	Exposure method	Dose administered (ng/g bw)	Concentration in tissues (ng/g ww)[a]	Effect
Eng et al. (2012) *Toxicol Sci*	BDE–99	Zebra finch *Taeniopygia guttata*	Nestlings orally exposed	0–173.8 ng/g bw/d for 21 d	332–14080 ng/g lw (plasma)	Males: No effect on total brain size, HVC, and RA Females: HVC smaller; No effect on total brain size and RA
Eng et al. (2014) *Ecotoxicology*	BDE–99	European starling *Sturnus vulgaris*	Nestlings orally exposed	0–173.8 ng/g bw/d for 21 d	332–14080 ng/g lw (plasma) (Eng et al., 2012) *Tox Sci*	No effect on brain mass or volumes of HVC, RA, and Area X

[a] The congeners in tissues are the same as those in the Chemicals column, unless otherwise noted.

within the AOP.

3.7. Growth and development

Normal growth and development of nestlings ensure successful survival and reproduction of those offspring in the future. Abnormal growth and development of sexual organs can reduce the competitive ability of an individual. Development *in ovo* may be so impaired that chicks fail to hatch, directly affecting the reproductive success of the parents. Normal growth and development, along with behaviour, are contingent upon several upstream physiological factors, including the HPT and HPG axes and retinol levels (see above).

In total, there were 15 studies on the effects of PBDEs and current-use FRs (i.e., DBE-DBCH, various OPEs) on growth and development when we include two papers that each examined two different species, as four separate studies (Berg et al., 2001; Bustnes et al., 2015; Crump et al., 2014; Currier et al., 2013, 2015; Eng et al., 2013a, 2013b, 2014a; Farhat et al., 2013; Fernie et al., 2006; Fernie and Marteinson, 2016; Rattner et al., 2013a; Winter et al., 2013) (Table 8). Of the ten studies that examined the effects of PBDEs on growth and development, six studies recorded an effect and four did not (Table 8). One of the six studies that recorded an effect on growth was a field study with a top predatory seabird, the great skua, in which PBDE exposure was associated with reduced growth in females, but not in males (Bustnes et al., 2015) (Table 8). However, supplementary feeding eliminated the effects of PBDEs (Bustnes et al., 2015). The other five studies that found an effect of PBDEs on growth were three laboratory studies with American kestrels (Fernie et al., 2006; Fernie and Marteinson, 2016; Rattner et al., 2013a) and two with zebra finches (Eng et al., 2013a, 2013b; Winter et al., 2013) (Table 8). We consider the study by Fernie et al. (2006) to have reported an effect of PBDEs, as the length of the tarsometatarsus and of the primary feather P9 were longer in exposed versus control chicks (significance at α=0.05). However, the difference in mass, with exposed chicks being heavier than controls, was significant at the 0.1 level. In one of the studies which reported an effect on growth, there was no effect on the first generation of zebra finches, but there was an effect on the second generation, suggesting that early exposure may lead to maternal effects on their offspring (Winter et al., 2013) (Table 8). The four studies that did not report an effect of PBDEs on nestling growth were laboratory studies with zebra finches (Currier et al., 2015; Eng et al., 2013a, 2013b), European starlings (Eng et al., 2014a), and common terns (*Sterna hirundo*) (Rattner et al., 2013a) (Table 8). Therefore, 66% of PBDE studies (n =6) that reported an effect on growth used predators as their study species, whereas no predatory species where included among the studies reporting no effect (n =4).

The comparison of developmental effects across multiple studies suggests that avian predators at the top of the food chain may be more susceptible than other species to PBDEs altering their growth and development, although consideration of the differences in exposure concentrations among avian taxa groups (Table 8), either in laboratory studies or as a result of feeding ecology and trophic position, should be taken into account. Two comparative studies to date, using the same concentrations of PBDEs (DE-71), demonstrated mixed results in terms of developmental differences among species: some growth parameters (i.e., hatchling body mass, crown-rump length) consistently showed no effects across four species (i.e., American kestrels, chickens, mallard ducks, common terns), while changes in other growth parameters (shorter humerus length in hatchlings; stunted development and small physical size relative to incubation age in embryos failing to hatch) were evident for the kestrels and not the chickens, mallards, or terns (McKernan et al., 2009; Rattner et al., 2009). More research is required to determine if there are species differences in growth and development in response to PBDEs, and to other FRs whose effects on avian growth are unknown.

Exposure to other FRs has also altered avian growth and development (Table 8). Out of 15 studies, including two papers that examined two types of FRs each counted as separate studies, 6 examined the effects of FRs other than PBDEs on growth and development. Studies that examined DBE-DBCH (Crump et al., 2014; Currier et al., 2013) or TBBPA (Berg et al., 2001) in the laboratory did not find effects on growth and development in chicken, Japanese quail, and zebra finches (Table 8). However, effects on embryonic growth and development were recorded in association with TMPP (Crump et al., 2014) and TCIPP and TDCIPP (Farhat et al., 2013) (Table 8). Specifically, chickens exposed to TMPP experienced reduced growth (i.e., shorter wings) and abnormal development (i.e., gastroschisis, twisted legs, and curled toes) (Crump et al., 2014). Exposure to TCIPP in chickens led to reduced tarsus length and liver somatic index whereas exposure to TDCIPP resulted in reduced mass, head-bill length, and gallbladder size (Farhat et al., 2013). In sum, although few studies examined developmental effects of FRs other than PBDEs, the available data suggest that some FRs impact avian growth and development warranting further research in the future.

Changes in an individual's growth and development can directly impact its survival, a measure of fitness, which in turn can produce changes at the level of avian populations within the AOP. Another component of fitness is reproduction, and the effects of various flame retardants on this measure are reviewed below.

3.8. Reproductive success

A direct measure of fitness, changes in reproduction can generate population-level effects. Measures of reproduction in this review include direct measures such as offspring survival or mortality, but also indirect measures related to offspring survival, such as pipping

success, eggshell thickness, and clutch size. Successful reproduction is mediated by several upstream effects, including the endpoints presented above, such as hormones, the brain and behaviour (previously discussed).

We report the effects of various FRs on eggshell thickness, an important measure of reproduction (Table 9). The thinning of eggshells of peregrine falcons due to their exposure to *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), a metabolite of DDT, led to their populations crashing in the 1950s and 1960s (Peakall, 1993). Is there any evidence that FRs are associated with the thinning of eggshells? Field studies did not find a correlation between eggshell thickness and PBDE or HBCD exposure in African penguins (*Spheniscus demersus*) (Bouwman et al., 2015) or ospreys (*Pandion haliaetus*) (Henny et al., 2009) (Table 9). However a laboratory study with American kestrels, a close relative of peregrine falcons, found that exposure to a PBDE technical mixture, DE-71, resulted in eggshell thinning (Fernie et al., 2009) (Table 9). Eggshell thinning may be especially sensitive to PBDE exposure relative to other FRs because other laboratory studies with American kestrels exposed to HBCD (Fernie et al., 2011) or β-DBE-DBCH (Marteinson et al., 2012b) did not find any evidence of eggshell thinning (Table 9).

In our review, we also report an effect on reproduction if clutch size, hatching success, and/or fledgling success were affected (Table 9). A total of 19 studies examined reproductive success measures other than eggshell thickness in relation to the exposure of birds to PBDEs, including three papers that examined multiple species and are considered separate studies (Table 9). Of these 19 studies, 12 found no effect (Bouwman et al., 2015; Bustnes et al., 2015; Currier et al., 2015; Eng et al., 2013a, 2013b, 2014a; Fernie et al., 2006; Gilchrist et al., 2014; McKernan et al., 2009 [2 species]; Rattner et al., 2013a [2 species]; Van den Steen et al., 2009) and 7 found a negative effect (Daso et al., 2015 [two species]; Fernie et al., 2009; Henny et al., 2009; Marteinson et al., 2010; McKernan et al., 2009; Winter et al., 2013) (Table 9). One study found a clear difference among species, with an impact on hatching success of American kestrels, but not of chickens or mallards (McKernan et al., 2009) (Table 9). Therefore, there are clear species differences in the reproductive effects of PBDEs. In the two studies that examined possible changes in hatching success relative to exposure to DBE-DBCH, one found no effect on chickens (Crump et al., 2014) and one found a negative effect on American kestrels (Marteinson et al., 2012b) (Table 9). The studies that examined exposure of TMPP (Crump et al., 2014), TCPP and TDCPP (Farhat et al., 2013), or HBCD (Fernie et al., 2011), found no effect on reproductive parameters such as clutch size, hatching success, and/or fledgling success (Table 9), although HBCD exposure reduced the egg size of American kestrels, with potential implications for reduced embryonic survival and hatching success (Fernie et al., 2011).

Overall, reproductive success was less frequently impacted than behaviour. That would be as expected if FRs primarily impact behaviour (either due to altered brain or altered hormone status or both), but individuals can compensate for altered behaviour. For instance, one member of the pair may compensate for the altered behaviour of the other member of the pair as was demonstrated in American kestrels by Marteinson et al. (2012a). Furthermore, one study on great skuas found an effect related to reproductive success (chick growth rate; included in the Growth and Development section) for unfed, but not fed, birds, showing that altered behaviour may not impact reproductive success when food is plentiful but may have an impact when food is limiting (Bustnes et al., 2015). Thus, we would expect that exposure to FRs would be a contributory factor to changes in reproductive success in some cases (e.g., wild birds that experience variations in food supply), but not in other cases (e.g., some laboratory studies with *ad libitum* food for avian models).

Because reproduction is a direct measure of an individual's fitness, changes in reproduction in response to FR exposure, are a critical component of the AOP. As with survival, changes in reproduction can produce subsequent population-level effects.

4. Species sensitivity

Variation in species sensitivity to chemicals has been demonstrated with polychlorinated dibenzo-*p*-dioxins and –furans in relation to the Ah-receptor (Head et al., 2008). Although the mechanism is unknown, there is some indication that variation in species sensitivity also exists with FRs. McKernan et al. (2009) concluded that kestrels were more sensitive to PBDEs, demonstrating stunted development and physical size relative to incubation age in embryos that failed to hatch, and decreased pipping and hatching success, in kestrels in relation to their exposure to the same concentrations of PBDEs that had no effects on survival or developmental endpoints in chickens or mallard ducks. Similarly, Rattner et al. (2013) concluded that common tern embryos, and possibly other tern species, are less sensitive to PBDEs than kestrel embryos, with only the kestrels demonstrating changes in multiple oxidative stress endpoints and reduced thyroid gland mass. Across all of the endpoints we examined in this review, we found that raptors were the most sensitive (Table 10). We recorded significant associations in 30–38% of studies across all endpoints for Galliformes, Passeriformes, and other species such as gulls, terns, and ducks (Table 10). However, for raptors, we recorded significant associations in 71% of studies (Table 10). Within each endpoint with data available for raptors, the proportion of significant associations were either the same or the highest in raptors except for steroids, which were highest in birds from the “other group”, which included ducks, gulls, and terns (Table 10). Galliformes appear to be the second most sensitive species, with this group ranking second within each of the three endpoints with data on this group of birds: thyroid system, growth and development, and reproduction (Table 10). Finally, based on current understanding (October 2016), our data suggest that Passeriformes and birds from the “other groups” appear to be less sensitive of these groups. This hypothesis should be considered in light of the varying trophic positions (wild birds) and exposure concentrations (laboratory studies) that occur across these studies. Nevertheless, our conclusions are consistent with the conclusions of the two previous comparative studies (McKernan et al., 2009; Rattner et al., 2013a). Since there has been somewhat limited research to date characterizing the effects of FRs on birds, further research, including additional comparative laboratory studies and studies with multiple species of wild birds, is required to test this hypothesis, and if validated, to elucidate the mechanism(s) underlying the potential differences in species sensitivity to FRs.

5. Overall synthesis and future directions

In the current review, we examined eight commonly measured endpoints in the context of determining possible effects on birds from exposure to various FRs. We found that some endpoints appear to be more sensitive to such FR exposure than others. Below, we synthesize our findings and recommend future directions for all of these endpoints.

Changes in mRNA expression *in vitro* and in *vivo* in the context of FR exposure have been measured in a wide array of genes, including those related to other endpoints in our review (thyroid system, sex steroids, lipid metabolism/neuron-related changes, and growth). At least 50% of studies recorded an effect on these genes, with thyroid-related genes being the most sensitive. However, nearly all of the studies included in the genomics section of our review were conducted on chickens, demonstrating the need to expand this technique to wild avian species.

The thyroid system is extensively studied in relation to characterizing potential *in vivo* effects of FRs on birds. Most research has addressed circulating thyroid hormones, with some studies also determining changes in the thyroid gland, notably hormone content, structure and/or function. However, when examined across species,

Table 7
Effects of various FRs on multiple behaviours of birds.

Paper	Chemical (s)	Species	Exposure method	Dose (ng/g bw)	Chemical concentration in tissues (ng/g ww)[a]	Effect
Eng et al. (2012) *Toxicol Sci*	BDE−99	Zebra finch *Taeniopygia guttata*	Oral	0–173.8/ d for 21 d	Plasma: 332–14080 ng/g lipid	Males: Exposed males sang less and fewer invited females in courtship Females: Response reduced to exposed males
Fernie et al. (2008) *Toxicol Sci*	DE−71[b]	American kestrel *Falco sparverius*	Adults exposed by diet	0–352 ng/g bw/d for 75 d	First egg of each pair: ∑PBDE: 3 −1131	Exposed pairs copulated less frequently Low exposed males generally ate more than control males High-exposure pairs performed more food transfers than low exposure or controls
Marteinson et al. (2010) *Environ Toxicol Chem*	DE−71[b] (HBCD unintentionally)	American kestrel *Falco sparverius*	Males exposed maternal transfer; paired one year later with unexposed females	Mother: 0–320 ng/g bw/d for 75 d	First laid egg (sibs become subjects): 3–1131	Reduced copulations, pair bonding behaviour, nest attendance; more flight displays in males
Marteinson et al. (2012a) *Chemosphere*	HBCD	American kestrel *Falco sparverius*	Adults pairs exposed by diet for 75 d	0–510 ng/g bw/d for 75 d	First laid egg of subjects: 0.7–180	Exposed pairs relative to controls: Reduced courtship vocalizations, bonding displays in females, and activity Reduced male parental behaviours; compensatory increase in females Lower incubation temperature
Marteinson et al. (2015) *Environ Toxicol Chem*	β-DBE-DBCH	American kestrel *Falco sparverius*	Adult pairs exposed by diet for 82 days	0–0.239 ng/g bw/d for 82 d	Not detected in eggs, plasma, fat, and liver	Increased copulations, courtship vocalizations and behaviours, male defense of nest box; No reduction in parental behaviours
Sullivan et al. (2013) *J Toxicol Env Heal A*	DE−71[b] (HBCD unintentionally)	American kestrel *Falco sparverius*	Pairs exposed by diet	0–320 ng/g bw /d for 75 d	∑PBDE: 3–1105 HBCD (unexpected): 0.002–16	Longer incubation periods, lower nest temperatures, lower incubation consistency
Verboven et al. (2009) *Anim Behav*	∑PBDE[c] ∑OH-PBDE[d] ∑MeO-PBDE[e]	Glaucous gull *Larus hyperboreus*	Correlations in free-ranging birds	N/A	In field, plasma (females, males): ∑PBDE: 7, 19.5 ∑OH-PBDE: 1.7, 2.2 ∑MeO-PBE: 0.7, 0.5	Negative correlation between ∑PBDE and mean nest temperature

[a] The congeners in tissues are the same as those in the Chemicals column, unless otherwise noted.
[b] BDE-17, −28, −47, −49, −66, −85, −99, −100, −138, −153, −154, −183, −209.
[c] BDE-17, −25, −28, −47, −49, −54, −66, −75, −77, −85, −99, −100, −116, −119, −138, −139, −140, −153, −154/BB153, −155, −156, −171, −180, −181, −183, −184, −190, −191, −196, −197, −201, −202, −203, −205, −206, −207, −208, −209.
[d] 6′-OH-BDE17, 4′-OH-BDE17, 6′-OH-BDE49, 2′-OH-BDE68, 6-OH-BDE47, 3-OH-BDE47, 5-OH-BDE47, 4′-OH-BDE49, 4-OH-BDE42, 6-OH-BDE90, 6-OH-BDE99, 2-OH-BDE123, 6-OH-BDE85, 6-OH-BDE137.
[e] 6′-MeO-BDE17, 4′-MeO-BDE17, 2′-MeO-BDE28, 4-MeO-BDE42, 5-MeO-BDE47, 6-MeO-BDE47, 3-MeO-BDE47, 4′-MeO-BDE49, 6′-MeO-BDE49, 2′-MeO-BDE68, 6-MeO-BDE85, 6-MeO-BDE90, 6-MeO-BDE99, 2-MeO-BDE123, 6-MeO-BDE137.

Table 8
Alterations in the growth and development of nestlings relative to their exposure to flame retardants.

Paper	Chemical	Species	Delivery method	Dose administered (ng/body weight)	Concentration in tissues (ng/g ww)[a]	Effects
Berg et al. (2001) *Environ Toxicol Chem*	TBBP A	Japanese quail *Coturnix japonica* Chicken *Gallus gallus domesticus*	Egg injection in yolk; Tissue collected 2 d before hatch	0–45000 ng/g egg 2, 20 ng/g egg for positive control Additional DES at 200 ng/g egg	Not reported	No effect on Mullerian duct and testis formation
Bustnes et al. (2015) *Plos One*	∑PBDE (BDE–47, 100, 153)	Great skua *Catharacta skua*	Plasma in the wild	N/A	Field (plasma): Females: 4.3–21.8 Males: 6.8–41.6	Reduced growth in females Interaction between supplementary feeding and ∑PBDE on growth rate (supplementary feeding eliminated effects of contaminants)
Crump et al. (2014) *Toxicol Appl Pharm*	DBE-DBCH TMPP	Chicken *Gallus domesticus*	Air-cell egg injections	DBE-DBCH: 0–54900 ng/g egg TMPP: 0–261400 ng/g egg	DBE-DBCH (20–22 d post injection): Liver: ND[b]–1069 Cerebral: ND–168 Yolk sac: ND–5604 TMPP: ≤0.04–32869	TMPP: Gastroschisis, short wings, twisted legs, and twisted toes DBE-DBCH: No deformities
Currier et al. (2013) *Bull Environ Contam Toxicol*	DBE-DBCH	Zebra finch *Taeniopygia guttata*	Injected in yolk of freshly laid eggs; Sacrificed 21 dph[c]	0, 2.3–94 ng/g egg	21 dph chicks: Not detectable in adipose, liver, or plasma In additional study: Incubation day 3: about 0.145–0.75 Incubation day 14: about 0.06–0.650	No effect on growth or survival of egg-injected chicks
Currier et al. (2015) *Bull Environ Contam Toxicol*	BDE–47	Zebra finch *Taeniopygia guttata*	Oral exposure for first 20 days dph	0–500 ng/g body weight/ d for 20 d	21 dph chicks: Plasma: 10–55 Liver: 750–14500 ng/g lw Adipose: 100–27000 ng/g lw	No effect on growth (mass and tarsus length)
Eng et al. (2013a) *Environ Toxicol Chem*	BDE–99	Zebra finches *Taeniopygia guttata*	Oral exposure for first 20 dph	0–173.8 ng/g bw/d for 20 d	332–14080 ng/g lw (plasma) (Eng et al., 2012 *Tox Sci*)	Decrease in BDE–99 during laying positively correlated with clutch mass
Eng et al. (2013a) *Environ Pollut*	BDE–99	Zebra finches *Taeniopygia guttata*	Oral exposure for first 20 dph	0–173.8 ng/g bw/ d for 20 d	332–14080 ng/g lw (plasma) (Eng et al., 2012 *Tox Sci*)	No effect on growth (mass)
Eng et al. (2014) *Ecotoxicology*	BDE–99	European starling *Sturnus vulgaris*	Oral dosing first 20 dph	0, 15.8, 173.8 ng/g body weight per day	332–14080 ng/g lw (plasma) (Eng et al., 2012 Tox Sci)	No effect on growth, bill colour change and testis and ovary development

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Table 8 *(continued)*

Paper	Chemical	Species	Delivery method	Dose administered (ng/body weight)	Concentration in tissues (ng/g ww)[a]	Effects
Farhat et al. (2013) *Toxicol Sci*	TCPP TDCPP	Chicken *Gallus domesticus*	Injection in air cell before incubation; Incubated until pipping (day 21–22)	TCPP (ng/g egg): < 0.2–51600 TDCPP (ng/g egg): < 0.06–45000	TCPP: Liver < 0.2–4.8 Cerebral hemisphere 0.7–5.8 Yolk sac < 0.2–10 TDCPP: Liver < 0.06–2.0 Cerebral hemisphere < 0.06–2.0 Yolk sac 0.9–100	TCPP (effects): Decrease in tarsus length and liver somatic index No effect on mass, head-bill length and gallbladder size TDCPP (effects): Reduced mass, head-bill length, and gallbladder size No effect on tarsus length and liver somatic index
Fernie et al. (2006) *J Toxicol Env Heal A*	∑PBDE (BDE–47, 99, 100, 153)	American kestrel *Falco sparverius*	Egg injection + dietary exposure of nestlings	Egg injection: 18700 ng/g egg Oral gavage: 15.6 ng/g bw/ d for 29 d	Whole chick homogenization at 36dph: 0.72–86.09	Exposed chicks (consumed more food than controls): Had longer tarsometatarsus and longer P9 at fledging No significant effect of mass
Fernie and Marteinson (2016) *Environ Toxicol Chem*	DE–71[d]	American kestrels *Falco sparverius*	Chicks exposed in ovo by maternal transfer	Mother exposed to 0–320 ng/g bw /d for 75 d	∑PBDE[e] in ovo: 0–1131 Will grow to be subjects; collected at 17–20 dph	Female chicks generally smaller
Rattner et al. (2013a) *Chemosphere*	DE–71 (congeners not listed)	American kestrels *Falco sparverius* Common tern *Sterna hirundo*	Air-sac egg injection; Chicks euthanized at hatching	Tern and kestrel: 0–20000 ng/g egg	Control eggs that were infertile or died early in incubation: Tern: < 52 Kestrel: < 4	Terns: no effect on bone length Kestrels: exposed eggs had shorter humerus length
Winter et al. (2013) *Environ Toxicol Chem*	BDE–99	Zebra finch *Taeniopygia guttata*	Egg injection (yolk sac, unincubated egg)	0–1000 ng/g egg	Not reported	First generation: no effect of mass and tarsus length Second generation: reduced mass from hatch to fledging

[a] The congeners in tissues are the same as those in the Chemicals column, unless otherwise noted.
[b] ND = not detectable.
[c] dph=days post hatch.
[d] BDE-28, –47, –100, –99, –154, –153, –183.
[e] BDE-17, –28, –47, –49, –66, –85, –99, –100, –138, –153, –154, –183, –190, –209.

Table 9
Changes in avian reproduction in the context of flame retardant exposure.

Paper	Chemical (s)	Species	Delivery method	Dose administered (ng/body weight)	Chemical concentration in tissues (ng/g ww)[a]	Effects
Berg et al. (2001) *Environ Toxicol Chem*	TBBPA	Japanese quail *Coturnix japonica* Chicken *Gallus gallus domesticus*	Egg injection in yolk; collected 2 d before hatching	0–45000 ng/g egg 2, 20 ng/g egg for positive control Additional DES at 200 ng/g egg	Not reported	High *in ovo* mortality
Bouwman et al. (2015) *Chemosphere*	∑PBDE[c] + HBCD	African penguin *Spheniscus demersus*	Eggs in the wild	N/A	Field (eggs): ∑PBDE: 0.18–20 HBCD: 0.10–0.13	No correlation between eggshell thickness and ∑PBDE or HBCD
Bustnes et al. (2015) *Plos One*	∑PBDE (BDE–47, 100, 153)	Great skua *Catharacta sku*	Plasma in the wild	N/A	Field (plasma): Females: 4.3–21.8 Males: average range 6.8–41.6	No correlation between egg loss and ∑PBDE in laying females
Crump et al. (2014) *Toxicol Appl Pharm*	TMPP	Chicken *Gallus gallus domesticus*	Air-cell egg injections	DBE-DBCH: 0–54900 ng/g egg TMPP: 0–261400 ng/g egg	DBE-DBCH: Liver: ND–1069 Cerebral: ND–168 Yolk sac: ND–5604 TMPP: Embryonic content: ≤0.04–32869	No effect on incubation time or pipping success for both chemicals
Currier et al. (2015) *Bull Environ Contam Toxicol*	BDE–47	Zebra finch *Taeniopygia guttata*	Oral exposure for first 20 dph[b]	0–500 ng/g body weight/ d for 20 d	21 dph chicks: Plasma: 10–55 Liver: 750–14500 ng/g lw Adipose: 100–27000 ng/g lw	No effect on breeding success or brood size
Daso et al. (2015) *Chemosphere*	∑PBDE[d]	Southern ground-hornbill *Bucorvus leadbeateri* - SGH Wattled Crane *Bugeranus carunculatus* - WC	Levels in eggshells in the wild	N/A	Field: SGH: 0–1743 WC: 52–123626	High hazard = potential reduced reproductive success and declining population SGH: 0.58–0.94 (exposed to low to moderate hazard) WC: 27.71–45.27 (exposed to high hazard)
Eng et al. (2013a) *Environ Pollut*	BDE–99	Zebra Finches *Taeniopygia guttata*	Oral exposure for first 20 dph	0–173.8 ng/g bw/ d for 20 d	332–14080 ng/g lw (plasma) (Eng et al. 2012 *Tox Sci*)	No effect on chick survival, clutch size, hatching success or fledging success
Eng et al. (2014b) *Sci Total Environ*	∑PBDE[e]	European starlings *Sturnus vulgaris*	Eggs at agricultural sites (wild); Condition in mothers in relation to her eggs	N/A	Field: ∑PBDE in eggs: 10.9 (2–307)	PBDE in eggs not associated with hatching and fledging success

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Table 9 *(continued)*

Paper	Chemical (s)	Species	Delivery method	Dose administered (ng/body weight)	Chemical concentration in tissues (ng/g ww)[a]	Effects
Farhat et al. (2013) *Toxicol Sci*	TCPP TDCPP	Chicken *Gallus gallus domesticus*	Injection in air cell before incubation; incubated until pipping (day 21–22)	TCPP (ng/g egg): < 0.2–51600 TDCPP (ng/g egg): < 0.06–45000	TCPP: Liver < 0.2–4.8 Cerebral hemisphere 0.7–5.8 Yolk sac < 0.2–10 TDCPP: Liver < 0.06–2.0 Cerebral hemisphere < 0.06–2.0 Yolk sac 0.9–100	No effect of both chemicals on pipping success
Fernie et al. (2006) *J Toxicol Env Heal A*	∑PBDE (BDE–47, 99 100, 153)	American kestrel *Falco sparverius*	Egg injection + exposure by diet of nestlings	Egg injection: 18700 ng/g egg Oral gavage: 15.6 ng/g bw/ d for 29d	Whole chick homogenization at 36dph: 0.72 –86.09	No effect on hatching or fledging success
Fernie et al. (2009) *Environ Sci Technol*	DE–71[f] HBCD (unintentionally)	America kestrel *Falco sparverius*	Adult pairs exposed by diet for 75 d; eggs laid collected and analyzed	0–352 ng/g bw/d for 75 d	Eggs laid by exposed pairs: ∑PBDE[g]: 3–1131 α-HBCD: 0.002–16	Thinner eggshell Reduced fertility, hatching, and fledging success
Fernie et al. (2011) *Environ Toxicol Chem*	∑HBCD (technical mix; α, β, γ)	American kestrel *Falco sparverius*	Adult pairs exposed by diet for 75 d	0–51 ng/g bw/ d for 75 d	Eggs laid by exposed pairs: 0.7–180.0	No effects on hatching or fledgling success, fertility, eggshell thickness
Gilchrist et al. (2014) *Sci Total Environ*	∑PBDE[h]	Tree swallow *Tachycineta bicolor*	*In ovo* concentration in the wild	N/A	Field (eggs): 83.6–590.1	No correlation between hatching, fledgling, and breeding success and *in ovo* PBDE
Henny et al. (2009) *Ecotoxicology*	∑PBDE[i]	Osprey *Falco sparverius*	*In ovo* concentration in the wild	N/A	Field (eggs): 97.7–897	Negative relationship between productivity and ∑PBDE concentration No correlation between PBDE and eggshell thickness
Marteinson et al. (2010) *Environ Toxicol Chem*	DE–71[j] HBCD (unintentionally)	American kestrel *Falco sparverius*	Males exposed *in ovo* by maternal transfer; paired one year later with unexposed females	Mother exposed to 0–320 ng/g bw /d for 75 d	3–1131 ng/g ww in first egg laid of dosed mother	Fewer eggs and smaller clutches
Marteinson et al. (2012b) *Environ Sci Technol*	DBE-DBCH	American kestrel *Falco sparverius*	Breeding pairs exposed by diet for 82 days	0–0.239 ng/g bw/ d for 82 d	After 4-weeks: subset of individuals sacrificed None detected in liver, fat, plasma, and egg samples	Fewer eggs, poorer egg fertility & hatch success; Fewer males produced No effect on eggshell thickness
McKernan et al. (2009) *Environ Toxicol Chem*	DE–71[k]	Mallard *Anas platyrhynchos* Chicken *Gallus gallus domesticus* American kestrels *Falco sparverius*	Air-cell egg administration; euthanized at day 1 ph	0–20000 ng/g egg	Chicken: ND–4930 Kestrel: 0.38–2800	Kestrel: decreased piping and hatching success No effect on chickens or mallards

(continued on next page)

Table 9 *(continued)*

Paper	Chemical (s)	Species	Delivery method	Dose administered (ng/body weight)	Chemical concentration in tissues (ng/g ww)[a]	Effects
Rattner et al. (2013a) *Chemosphere*	DE–71 (congeners not listed)	American kestrels *Falco sparverius* Common tern *Sterna hirundo*	Air-sac egg injection; subjects euthanized at hatching	Tern and kestrel: 0–20000 ng/g egg	Infertile or dead eggs: Tern: < 52 Kestrel: < 4	Terns and kestrels: No effect on embryonic survival, pipping, and hatching success
Van den Steen et al. (2009) *Environ Pollut*	∑PBDE[l]	European starling *Sturnus vulgaris*	Silastic tubes in adults over breeding (at least 60 days)	Approx: 0–144 ng/g bw/d for 60 d	∑PBDE in serum: 0.151–23.4 ng/ml	No effect on clutch size or motivation to incubate
Winter et al. (2013) *Environ Toxicol Chem*	BDE–99	Zebra finch *Taeniopygia guttata*	Egg injection (yolk sac, unincubated egg)	0–1000 ng/g egg	Not reported	*In ovo* exposed birds laid smaller clutches No difference in hatching success and brood size; No difference in parameters in second or third generation

[a] The congeners in tissues are the same as those in the Chemicals column, unless otherwise noted.
[b] dph=days post hatch.
[c] BDE-47, –99, –100, –153, –154, –183, –206, –207, –208, –209.
[d] BDE-28, –47, –100, –99, –154, –153, –183, –209.
[e] 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, 209.
[f] BDE-17, –28, –47, –49, –66, –85, –99, –100, –138, –153, –154, –183, –209.
[g] BDE-99, –153, –100, –154, –47, –138, –49, –183, –209, –28, –17.
[h] BDE-99, –47, –100, 153, 154, 209.
[i] BDE-17, –28, –47, –49, –66, –85, –99, –100, –138, –153, –154, –183, –190, –209.
[j] BDE-17, –28, –47, –49, –66, –85, –99, –100, –138, –153, –154, –183, –209.
[k] BDE-17, –28, –47, –49, –66, –85, –99, –100, –153, –154.
[l] BDE-28, –47, –49, –66, –85, –99, –100, –153, –154, –183.

Table 10
An assessment of potential differences among avian species in their sensitivity to the effects of flame retardants, within and across endpoints examined. The percentage represents the proportion of studies in which at least one significant effect was reported for a given endpoint. Numbers below percentages represent the number of studies in which this endpoint was studied. The "other groups" included phylogenetically varied species from the following seven orders: Anseriformes, Charadriiformes, Coraciiformes, Gruiformes, Pelecaniformes, Procellariiformes, and Sphenisciformes.

Order	Thyroid system	Steroids	Retinol	Brain	Behaviour	Growth and development	Reproduction	TOTAL
Domesticated species (Galliformes)	29% 7	n/a 0	n/a 0	n/a 0	n/a 0	50% 6	29% 7	*35%* *20*
Raptors (Accipitriformes, Falconiformes)	55% 11	60% 5	100% 4	n/a 0	100% 4	100% 3	63% 8	*71%* *35*
Songbirds (Passeriformes)	20% 5	0% 1	n/a 0	50% 2	100% 1	40% 5	17% 6	*30%* *20*
Other groups	20% 5	100% 4	0% 4	n/a 0	100% 1	33% 3	25% 8	*38%* *24*

effects on circulating thyroid hormones are frequently not found and this is not surprising given the tight regulation of their concentrations for multiple physiological systems supporting survival. The component of the thyroid system that is most impacted by various FRs is the thyroid gland, and most likely, glandular structure and its ability to produce and/or release T_4 into circulation. In the context of PBDE contamination, most studies examined circulating T_3 and T_4 only, and these concentrations seem to be most affected at the nestling stage. Thus, future studies examining potential thyroid disruption with respect to FRs should include measurements of circulating hormones concurrently with measurements of the thyroid gland (e.g., using histology, the TSH challenge, and/or glandular T_4 concentrations). Various thyroid-related hepatic deiodinases and conjugation enzymes should also be included in the analyses. When possible, research should conduct such assessments of the various components involved with thyroid function during the nestling stage. Concurrent assessment of these multiple endpoints is highly recommended in future studies. There may be no changes in circulating T_3 and T_4 concentrations, but concurrent changes in glandular function or Phase I or II enzyme activity (e.g., Fernie et al., 2015). Future studies should also seek to characterize and develop an understanding of the molecular mechanisms involved with avian thyroid function that also may be altered by FRs, particularly in non-domestic avian species.

Retinol levels, both in the blood and in the liver, do not seem to be consistently sensitive to FR exposure across chemicals, species, or age, although retinol concentrations in studies with raptors appear to have some sensitivity to FR exposure. More research is required to support this conclusion. Depending on the FR, steroid hormones seem to be a promising endpoint especially when research assesses potential effects of a FR on multiple hormones simultaneously. Therefore, we recommend future research focus more on multiple steroids concurrently in determining the potential toxicity of FRs.

Given that behaviour was a commonly impacted endpoint, we recommend that future studies concentrate on behaviour, notably behaviours throughout the reproductive period. Because behaviour is a consequence of physiological reactions in the brain, partly mediated via hormonal cascades, we would recommend examining multiple endpoints along the hormonal cascade, as well as the brain itself in conjunction with related behaviours, to better understand the mechanisms involved in determining how a FR affects birds.

We recommend that future studies continue to measure changes in growth, development, and reproduction, because these endpoints are closely linked to reproductive success and avian populations. Moreover, we found that they are sensitive endpoints to FR exposure across multiple species and multiple FRs. Notably, growth and development was the second most sensitive endpoint, after behaviour, based on the number of studies that found at least one significant effect within a given endpoint (Table 10). Future research should link biochemical disruptions and changes in the brain to ecologically relevant endpoints, such as behaviour, growth and development, and reproduction. Although potential effects of FRs on the song control system in the brain of songbirds has been the focus of research so far, other brain regions that are present in the brain of non-passerines should be assessed, such as the hippocampus, a region responsible for spatial memory processing. Finally, we recommend the development and use of molecular techniques supporting these commonly measured *in vivo* endpoints for non-model avian species. In sum, endpoints spanning as broad of a range in the AOP as possible should be concurrently measured within studies. Such connections are essential to link FR exposure with reduced fitness and population-level effects on birds.

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