THE EFFECTS OF A DIETARY OR *IN OVO* EXPOSURE TO THE PENTABROMINATED DIPHENYL ETHER MIXTURE, DE-71, ON RETINOL AND THYROID HORMONES IN CAPTIVE AMERICAN KESTRELS (*FALCO SPARVERIUS*)

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CONTRIBUTIONS OF CO-AUTHORS

This thesis contains two manuscripts that will be submitted to refereed journals for publication. I am primary author for both manuscripts, having made the most substantial contribution, which includes analyses of the data and preparation of the manuscripts. My co-supervisors Kim J. Fernie and David M. Bird will be second and last author, respectively. In addition, Ian J. Ritchie, Robert J. Letcher, and J. Laird Shutt will be third, fourth and fifth author, respectively. Kim and J. Laird Shutt were the principal investigators of this research under the auspices of Environment Canada; both assisted in the development of my research ideas, and provided guidance and funding for myself and the overall research via Environment Canada. Both Kim and David contributed heavily to the editing of this thesis. In addition, David also provided financial support via his NSERC grant and the Avian Science and Conservation Centre. Ian J. Ritchie has over 30 years of experience at the Avian Science and Conservation Study where this study took place. His experience was invaluable and this study would not have been possible without his assistance. Robert J. Letcher provided intellectual input into the research, supervised the analysis of the *in ovo* PBDE congeners in his laboratory at the National Wildlife Research Center, and provided the resulting data and analytical information, without which much of the findings would not have been possible.

ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are one type of brominated flame retardant commonly used in industrial and domestic items. Their lipophilic and persistent properties and global occurrence in wildlife are cause for concern, especially for those at the top of the food chain, such as raptorial birds. This study consisted of dietary or *in ovo* exposure of captive American kestrels (*Falco sparverius*) to one of two environmentally relevant levels (high and low exposures) of the pentaBDE mixture, DE-71. Maternal retinol levels were reduced in the low-exposure group after three weeks of exposure and were associated with fewer hatchlings and increasing *in ovo* BDE-17 concentrations. The *in ovo* exposure affected the retinol levels of nestling kestrels and altered their thyroid hormones and the functioning of their thyroid system. These parameters were associated with *in ovo* concentrations of various BDE congeners, body mass, and feather growth. These findings are consistent with the reduced reproductive success of these birds and the altered growth of their young.

RÉSUMÉ

Les éthers de diphényle polybromé (EDPB) sont un type d'ignifuge bromé couramment utilisé dans les produits industriels et domestiques. Leurs propriétés lipophiles et persistantes ainsi que leur distribution mondiale dans la faune sont causes de préoccupation, surtout à l'endroit des espèces qui occupent le sommet de la chaîne alimentaire, tels que les oiseaux de proie. Cette étude comporta une exposition par voie alimentaire ou *ovo* de crécerelles d'Amérique captives (*Falco sparverius*) à l'un parmi deux niveaux écologiquement pertinents (expositions haute et faible) du mélange pentaEDB, DE-71. Les niveaux maternels de rétinol ont été réduits dans le groupe à faible exposition après trois semaines d'exposition et ont été associés à une réduction dans le nombre de poussins et à des concentrations *ovo* croissantes de EDB-17. L'exposition *ovo* a affecté les niveaux de rétinol chez les crécerelles juvéniles et a altéré leurs hormones thyroïdiennes et le fonctionnement du système thyroïdien. Ces paramètres ont été associés aux concentrations *ovo* de divers congénères EDB, à la masse corporelle et à la croissance des plumes. Ces résultats sont en accord avec le succès reproducteur réduit de ces oiseaux et l'altération de la croissance chez leur progéniture.

CHAPTER 1 – Literature review: An introduction to polybrominated diphenyl ethers (PBDEs) and the thesis research rationale, aim and objectives

Use

The risk and damage posed by fires to people and property can be extensive and life-threatening, but the employment of flame retardant chemicals has reduced the impact of fire over the past 30 years (Costa and Giordana 2007). Flame retardants do not eliminate the occurrence of fires, but impede the spreading of flames during a fire, thereby allowing more time to secure property and reduce the risk of death. While some flame retardants contain phosphorus or nitrogen (Birnbaum and Staskal 2004), brominated flame retardants (BFRs) have been found to be less costly and easier to manufacture (Rahman et al. 2001). BFRs are further classified into three categories, one of which, i.e., polybrominated diphenyl ethers (PBDEs), is the focus of this research.

PBDEs come in three major commercial mixtures: pentaBDE, octaBDE, and decaBDE. In 2001, the global market demand of these three mixtures was 11%, 6%, and 83%, respectively (La Guardia et al. 2006). The former two mixtures, however, were banned by the European Union in 2004 (Costa and Giordana 2007). In North America, the U.S. producer voluntarily stopped production of the pentaBDE and octaBDE mixtures in 2004 (Renner 2004) and their use was also banned in some states (Costa and Giordana 2007) and in Canada (Canada Gazette 2006) in 2006.

PBDEs have been used in a variety of products including commonly found industrial, office and household items such as clothing, carpet, and electronic appliances (Rahman et al. 2001). The pentaBDE mixture was used primarily in polyurethane foams, e.g., furniture, the octaBDE mixture in plastics, e.g., small appliances, and the decaBDE mixture in larger electronics, e.g., computers (Costa and Giordana 2007). PBDEs are therefore in widespread use in products that have a global distribution.

Chemical structure and properties

There are theoretically 209 possible PBDE congeners based on variations in the position and number of bromines. However, the actual number of congeners found in the technical mixtures is much lower, likely as a result of the instability of some of them (Birnbaum and Staskal 2004). The main congeners present in the three major PBDE commercial mixtures are as follows: 1) the pentaBDE mixture, DE-71, contains mainly tetra-, penta- and hexa-BDE congeners, specifically BDE-99 (49%), BDE-47 (38%), BDE-100 (13%), and BDE-153 (5%); 2) the octaBDE mixture, DE-79, contains hepta-, octa- and nona-BDE congeners, specifically BDE-183 (42%), BDE-197 (22%), BDE-207 (12%), and BDE-196 (11%); and 3) the decaBDE mixture, Saytex 102E, is almost entirely composed of the decaBDE congener BDE-209 (97%) (La Guardia et al. 2006). Table 1 contains the classification, e.g., pentaBDE, of the main BDE congeners mentioned above for these commercial mixtures.

PBDEs are additive flame retardants, meaning that they are mixed with, but not bound to, other polymers to make final products (Birnbaum and Staskal 2004). They are persistent and stable in nature with an ability to withstand degradation by acids or bases, heat, light, and reducing or oxidizing agents (Rahman et al. 2001). They may, however, have the ability to debrominate under UV light when they are dissolved in organic solvents, with the lower brominated congeners degrading more slowly (Birnbaum and Staskal 2004), i.e., decaBDEs have been found to debrominate to lower brominated congeners (Soderstrom et al. 2004). PBDEs have also shown high lipophilicity, with high K_{ow} values (Rahman et al. 2001), low solubility in water, high boiling points (Darnerud et al. 2001), and high vapour pressures, which increase as bromination decreases (Birnbaum and Staskal 2004). The lipophilic property of PBDEs is critically important as this allows for their persistence and bioaccumulation in biota, which is of particular concern to organisms at the top of the food chain such as birds of prey (Law et al. 2003).

Release and occurrence in the environment

PBDEs can enter the environment through effluents and flue gases from their production factories (Watanabe and Sakai 2003). They can also be released into the

environment through waste disposal, where PBDE-containing products in landfills leach PBDEs into the environment (Rahman et al. 2001). PBDEs are added to the products and are not chemically bound. This allows for their slow release from the products into the environment (McDonald 2002). There is also evidence that PBDEs are transported in air, either in gas or particle phases (Strandberg et al. 2001). Finally, they can also enter the environment indirectly through the food chain via diet (Watanabe and Sakai 2003).

PBDEs have been found in abiotic samples worldwide; for reviews on such findings see de Wit (2002) and Hale et al. (2003) for North America, and Wang et al. (2007) for East Asia. The solubility of PBDEs in air and water has been predicted to decrease with increasing bromination (Palm et al. 2002) with PBDEs mainly separating into soil and sediment (Palm et al. 2002, Gouin and Harner 2003). PBDEs have, in fact, been shown to become strongly absorbed in soil and sediment, resulting in the congeners found being highly representative of the commercial mixtures (Birnbaum and Staskal 2004). The high vapour pressure of the lower brominated congeners result in their prevalence in air samples (Birnbaum and Staskal 2004). BDE-47, -99, and -209 are generally the predominant congeners found in air and sediment samples around the world in terms of concentration (de Wit 2002, Wang et al. 2007).

To date, all brominated compounds have been predicted to have a similar persistence in the environment (~220 d), which is of concern due to the potential of annual carry-over (Palm et al. 2002). However, Gouin and Harner (2003) predicted increasing half-lives, and therefore persistence, for all environmental components, e.g., air, soil, with increasing bromination. PBDEs have also been suggested to have a moderate long-range transport (480-1200 km) compared to other compounds (Palm et al. 2002), where increased transport occurred with decreasing bromination (Gouin and Harner 2003).

Interestingly, PBDEs have been shown to be sensitive to fluctuating temperatures, i.e., decreased temperatures resulted in a reduction of PBDEs in the air (Gouin and Harner 2003). In general, air sample concentrations are higher in the summer (Wang et al.

2007). Similarly, PBDE concentrations are also higher in city centres compared to rural areas (Wang et al. 2007). For example, concentrations of PBDEs in air samples above Chicago were 5-10 times greater than in nearby rural areas (Strandberg et al. 2001). Air samples are, however, typically highest in electronics recycling plants (Wang et al. 2007).

PBDEs in sediment also have much higher concentrations downstream compared to upstream of a source, i.e., plastics industry (de Wit 2002, Wang et al. 2007). Of several samples taken along the River Viskan in Sweden, the lowest concentrations of PBDEs were found upstream of industry and their concentrations increased further downstream and as more industries were passed (Sellstrom et al. 1998).

The North American abiotic environment demonstrates higher concentrations of PBDEs than those of either Europe or Eastern Asia (Hale et al. 2003, Wang et al. 2007), most likely due to a historically higher demand and use of PBDEs in North America compared to the other continents (Rahman et al. 2001, Birnbaum and Staskal 2004).

PBDE findings and trends in wildlife

PBDEs have been detected in many wildlife species throughout the world; see reviews by Law et al. (2003) and Hites (2004). They have been found in marine and freshwater invertebrates, i.e., blue mussels (*Mytilus edulis*): Wang et al. 2009 and zebra mussels (*Dreissena polymorpha*): Covaci et al. 2005, marine crustaceans, i.e., spider crabs (*Maja squinado*): Bodin et al. 2007, bivalves, i.e., oysters (*Crassostrea gigas*): Moon et al. 2007, frogs (*Rana temporaria*): Ter Shure et al. 2002, and both freshwater and marine fish, i.e., whitefish (*Coregonus* sp.) and rainbow trout (*Oncorhynchus mykiss*): Zennegg et al. 2003; carp (*Cyprinus carpio*) and largemouth bass (*Micropterus salmoides*): Rice et al. 2002, eel (*Anguilla anguilla*) and gibel carp (*Carassius auratus gibel carpio*): Covaci et al. 2005. Investigations of PBDEs in top predatory birds have been fairly extensive, with findings in marine, freshwater and terrestrial environments (marine: northern fulmars (*Fulmarus glacialis*): Karlsson et al. 2006, white-tailed sea eagles (*Haliaeetus albicilla*): Herzke et al. 2005, guillemots (*Uria algae*): Sellstrom et al. 2003, glaucous gulls (*Larus hyperboreus*): Herzke et al. 2003; freshwater: cormorants

(*Phalocrocorax carbo*): Law et al. 2002, Watanabe et al. 2004, grey herons (*Ardea cinerea*) and great crested grebes (*Podiceps cristatus*): Jaspers et al. 2006; terrestrial: sparrowhawks (*Accipiter nisus*) and common buzzards (*Buteo buteo*): Voorspoels et al. 2007, merlins (*Falco columbarius*), golden eagles (*Aquila chrysaetos*) and goshawks (*A. gentilis*): Herzke et al. 2005, and little owls (*Athene noctua*): Jaspers et al. 2005). PBDE levels have also been detected in passerine birds, i.e., great tits (*Parus major*): Dauwe et al. 2006, van den Steen et al. 2006, but to a lesser extent. Mammals have also been investigated in both marine and terrestrial settings (marine: Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*): Ramu et al. 2005; harbor seals (*Phoca vitulina*): She et al. 2002; harbour porpoises (*Phocoena phocoena*: Law et al. 2002; terrestrial: moose (*Alces alces*), European roe deer (*Capreolus capreolus*), and lynx (*Lynx lynx*): Mariussen et al. 2008, wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*): Voorspoels et al. 2007, and red foxes (*Vulpes vulpes*): Voorspoels et al. 2006) with higher levels found in marine mammals. PBDEs have also been detected in humans worldwide with varying concentrations in blood and breast milk samples, i.e., U.S.: Schecter et al. 2003; U.K.: Kalantzi et al. 2004; China: Bi et al. 2006.

Biomagnification occurs when a substance, such as PBDEs, increases in concentration up the food chain due to either the persistence of the substance, the energetics of the food chain and/or the lack of internal degradation or excretion of the substance. PBDEs have been found in wildlife in locations as remote as the Arctic (see review: de Wit et al. 2006) and that food web is only one of several where PBDEs have biomagnified. Biomagnification of PBDEs has also occurred in freshwater food webs (Law et al. 2006). Biomagnification has been observed in predatory birds in terrestrial food chains but not for the mammals because they may have the ability to metabolize PBDEs to a greater extent (Voorspoels et al. 2007). In fact, some of the highest levels of PBDEs found in the wild have occurred in top predatory birds: common kestrels (*F. tinnunculus*; max. level 40 900 ng/g lw in liver: Chen et al. 2007), sparrowhawks (max. level 64 000 ng/g lw in liver: Jaspers et al. 2006), great blue herons (*A. herodias*), double-breasted cormorants (*P. auritus*), and osprey (*Pandion haliaetus*) (> 20 000 ng/g lw in the

eggs; Elliot et al. 2005). The highest recorded levels ever found were in peregrine falcon (*F. peregrinus*) eggs in urban environments in California (max. level 52 ppm; Cone 2008).

To date, the most prevalent congeners found in wildlife are BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183 (Mikula and Svobodova 2006). The lower brominated congeners are more persistent and more likely to bioaccumulate, as reflected by their increasing concentrations in biota higher in the food chain (de Wit 2002). These lower brominated congeners are also, in general, more toxic than the higher congeners (Birnbaum and Staskal 2004). The retention factors and half-lives of four BDE congeners (BDE-47, -99, -100, 153) were determined to be 7.8-45.3% and 5.6-44.7 d, respectively, in juvenile American kestrels (*F. sparverius*) with adult PBDE half-lives expected to range from 72 to 572 d (Drouillard et al. 2007). These half-lives contrast with the prediction that all brominated compounds have a similar persistence in the environment (~220 d; Palm et al. 2002).

The tissue distribution of PBDEs has been reviewed by Hakk and Letcher (2003) with fat, liver, kidney, and muscle being large accumulators compared to other internal organs. Due to their high lipid concentrations (usually 10%), avian eggs also accumulate PBDEs through maternal transfer and thus their levels reflect well the female's contaminant burdens (Dauwe et al. 2006). Transfer of PBDEs can also occur through the placental wall to the fetus as well as through the mother's milk in mammals (see review: Costa and Giordano 2007).

Temporal trends in wildlife have also been assessed for PBDEs. Levels in the Arctic have been exponentially increasing in ringed seals (*P. hispida*), with penta- and hexa-BDEs increasing the most rapidly with doubling times of 4.7 and 4.3 years, respectively (1981-2000; Ikonomou et al. 2002). PBDE levels in peregrine falcons have also been increasing in Greenland with an increase of approximately 10% every year (1986-2003; Vorkamp et al. 2005). Cormorants and herons in British Columbia also experienced exponential increases of PBDEs with a doubling time of 5.7 years (1979-

2002; Elliot et al. 2005). Finally, Great Lakes herring gulls (*L. argentatus*) have doubling times of 2.6-3.1 years for PBDEs (1981-2000; Norstrom et al. 2002), although more recently, PBDE levels have decreased or are showing non-increasing trends in an assessment of the same sites in 2004 (Gauthier et al. 2007).

Effects of PBDEs on mammalian and avian species

The implications of discovering such levels of PBDEs in wildlife are currently not fully understood. Relatively few studies have detailed their physiological effects. The following sections will focus on findings relating to reproductive and endocrine changes associated with PBDEs.

Reproductive effects

Reproductive success is reliant on a number of properly timed behaviours, e.g., courtship, copulations, incubation, etc., and appropriate hormonal regulation to ensure that young are successfully produced. Reproductive success is sometimes measured by the number of young produced. Several controlled experimental studies have involved different species in which the administration of PBDEs resulted in fewer young produced compared to controls. For example, the administration of BDE-99 to pregnant mice led to a reduction in the litter size compared to controls (Branchi et al. 2002). In contrast, litter size in rats was not affected by exposure to DE-71 (Zhou et al. 2002). Ranch mink (*Mustela vison*) also experienced total reproductive failure, i.e., 100% of dams failed to whelp, when exposed to DE-71 but only at the highest exposure level (2.5 ppm; Zhang et al. 2009). Another study involving the exposure of a top avian predator, i.e., the American kestrel, to DE-71 also resulted in a reduced reproductive success, i.e., fewer hatchlings and fledglings (Fernie et al. 2009), as did an *in ovo* exposure of male kestrels to DE-71 paired with unexposed females, i.e., fewer fertile eggs and smaller clutch sizes (S. Marteinson, upubl. data). A decrease in brood sizes of wild peregrine falcons was also correlated with increasing concentrations of PBDEs (Johansson et al. 2009). Finally, PBDEs were associated with reduced nest productivity in wild ospreys but only when PBDEs exceeded 1000 ng/g ww in the eggs (Henny et al. 2009).

Other components of the reproductive cycle prior to brood rearing, i.e., courtship and incubation phases, can also affect the reproductive output of birds. American kestrels exposed by diet and *in ovo* to DE-71 demonstrated altered courtship behaviours, e.g., fewer copulations, resulting in a reduction in the strength of the pair bond (Fernie et al. 2008, S. Marteinson, upubl. data). In the incubation phase, embryonic development is reliant on appropriate incubation temperatures. It has been suggested that incubation temperatures may be affected by PBDE exposure, i.e., a decrease in incubation temperatures as PBDE concentrations increased in glaucous gulls (Verboven et al. 2009). In addition, the hormone responsible for the onset and maintenance of incubation, prolactin, was found to decrease in incubating male glaucous gulls as the concentration of PBDEs in their system increased (Verreault et al. 2008). Inappropriate courtship, i.e., lack of a pair bond, and decreased attention to egg incubation as a result of PBDE exposure could in part explain the observed reduced reproductive success observed in other avian studies (Fernie et al. 2009, Henny et al. 2009, Johansson et al. 2009, S. Marteinson, upubl. data).

Successful hatching in birds is a function of appropriate embryonic temperatures, as well as the environment in which the embryo is developing. Eggs were smaller (volume, mass) and had thinner eggshells when American kestrel females were exposed by diet to DE-71 (Fernie et al. 2009), perhaps representing a reduction in the nutrition of the eggs for the developing embryo. Greater egg weight loss by mid-incubation occurred in the PBDE-exposed kestrels compared to the controls, which suggests an abnormal gas exchange (Fernie et al. 2009). McKernan et al. (2009) found that American kestrel embryos were more sensitive to PBDE administration via the egg's air cell than either chickens (*Gallus gallus*) or mallards (*Anas platyrhynchos*). Their kestrel embryos also had both decreased pipping and hatching success (McKernan et al. 2009).

The courtship and incubation behaviours of birds, as well as the successful production of embryos, are under hormonal control. While there is limited information on the disruption of sex hormones by PBDEs, they have now been classified as "endocrine disruptors". Some PBDEs display estrogen-like effects (Meerts et al. 2001) as well as

anti-androgenic effects (Stoker et al. 2005). A disruption of these hormones, which are critical to successful reproduction, may explain the reported negative impacts on breeding success. PBDEs may also affect the reproductive tract. For example, rodents exposed to DE-71 experienced a decrease in the size of some of their reproductive organs, i.e., seminal vesicle and ventral prostate weights (Stoker et al. 2004). Reduced sperm counts were also reported in rodents developmentally exposed to BDE-99 (Kuriyama et al. 2005). Finally, another gestational study with BDE-99 and rodents showed a decrease in sex steroids and a slight acceleration of puberty in males, whereas the females experienced a delay in the onset of puberty and a decline in their ovarian follicles (Lilienthal et al. 2006).

Thyroid hormones

The thyroid system is critical for reproduction, metabolism, thermoregulation, and growth (McNabb 2000). It is one of the primary targets of PBDEs. The structure of some PBDE congeners and their metabolites is similar to that of thyroxine (T_4), one of the two thyroid hormones (McDonald 2002), and thus, they out-compete T_4 for the transthyretin (TTR) carrier resulting in the disruption of this system. The structural requirements for hydroxylated PBDE binding affinity to TTR are similar to those of hydroxylated PCBs: those with a hydroxy group in the *meta* or *para* position with one of more adjacent halogen substitutions show more of an affinity for TTR (human) binding, most likely due to their similarity to T_4, as well, the degree of bromine substitution plays a key role in the level of TTR binding affinity where those with a greater degree of bromination show a higher binding affinity for TTR (Meerts et al. 2000). Competition for T_3-TTR (piscine) binding has also been observed for PBDEs and hydroxylated PBDEs with greater affinities for the latter and those with a smaller degree of bromination (Morgado et al. 2007). Given these structural similarities and greater binding affinities, hydroxylated PBDEs can outcompete and displace T_4 and T_3 from the TTR protein carrier in the blood.

Both thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), have been investigated regarding potential impacts of PBDEs. Fernie et al. (2005) addressed PBDE (DE-71) exposure (*in ovo* and developmentally) in juvenile American kestrels and found

that thyroid function was altered by the DE-71 exposure, resulting in a reduction of the T_4 levels. In rodents, PBDEs have been shown to reduce thyroid hormone levels in fetuses, offspring, and dams with T_4 again showing reductions as a function of the PBDE exposure (Hallgren et al. 2001; Zhou et al. 2001, 2002). PBDE metabolites have also been found to compete with T_4 for binding sites to the protein responsible for their transport, i.e., transthyretin (TTR) (Meerts et al. 2000), indicating a mechanism for the reduction of T_4 levels. A reduction in T_3 levels has also been observed in the offspring of ranch mink exposed to DE-71 (Zhang et al. 2009). Lambs exposed gestationally to BDE-47 also experienced reductions of both thyroid hormones (Abdelouahab et al. 2009).

Retinol

Retinol, the animal form of vitamin A, is a physiologically important nutrient required for reproduction, growth, and vision (Ganguly 1989). Vitamin A is stored mainly in the liver as an ester that is hydrolyzed to form retinol (Ganguly 1989). Retinol then binds to its carrier proteins, i.e., TTR and retinol binding protein (RBP), and is released into the circulation as needed (Ganguly 1989). Its disruption due to PBDE exposure has also been examined, although less extensively than the thyroid system. Dietary PBDE exposure has been found to reduce hepatic vitamin A levels in rats and mice (Hallgren et al. 2001). According to Fernie et al. (2005), *in ovo* (egg injection) coupled with developmental exposure of juvenile American kestrels to PBDEs (DE-71) led to marginal reductions in plasma retinol and hepatic retinol concentrations. In contrast, plasma and hepatic retinol levels in domestic ducks were not affected by *in ovo* exposure to BDE-99 (Murvoll et al. 2005). There are also cases, however, in which retinol levels were altered due to PBDE exposure only in conjunction with a reduced retinol diet (Ellis-Hutchings et al. 2006, 2009).

As mentioned above, retinol is only released from the liver when needed and is transported in the blood to the target cells by TTR and RBP (Ganguly 1989). Similar to T_4 above, the ability of PBDE metabolites to compete for TTR-binding sites (Meerts et al. 2001) is one possible mechanism of action whereby PBDE exposure reduces retinol levels. An alternative mechanism could be increases in uridine diphosphate-glucuronosyl

transferase (UDPGT) activity caused by PBDE exposure in laboratory rodents (Zhou et al. 2001, 2002). This allows for a greater clearance of conjugated retinol from the body, making it unavailable for reabsorption in the kidney.

Project rationale, aim and objectives

PBDEs are widely used BFRs. They have dispersed globally and are found in a variety of environments. The implications of detecting PBDEs in various species of wildlife are currently not fully understood as there are few studies detailing their physiological effects. Validated tests are paramount for addressing these knowledge gaps, especially for avian species inhabiting areas with higher concentrations of PBDE compounds.

The thyroid system and vitamin A (retinol) are physiologically important for all vertebrates, including avian species. Retinol and the thyroid hormones are important as they play a critical role in the appropriate growth and maturation of young birds. Retinol is also important for embryonic development and hence, hatching success. Determining the effects of PBDEs on top predators, i.e., raptorial birds, where bioaccumulation of PBDEs occurs most acutely, is crucial. PBDEs have the potential to reduce the reproductive success and alter the growth of these high trophic level species, which could result in population declines in the wild. With the persistence of PBDE contaminants in our environment, a more in-depth understanding of the effects of PBDEs on wildlife is vital for wildlife conservation.

The overall aim of my M.Sc. thesis research was to examine the effects of a dietary exposure of environmentally relevant levels of the pentaBDE mixture, DE-71, on retinol levels in breeding birds and the retinol levels and thyroid system of their *in ovo* exposed nestlings. I used a small falcon, the American kestrel, as a model for a high trophic level avian species. The American kestrel has proved to be an ideal candidate to study the effects of PBDEs (Fernie et al. 2005, 2008, 2009) and its value as a laboratory animal is well-documented (Bird 1982).

Pairs from a captive colony of American kestrels with known pedigree (Bird 1982) were exposed by diet to PBDEs for approximately 75 days prior to pairing and up until hatching time. The study consisted of a control group and two exposure groups, i.e., high and low, which were administered environmentally relevant concentrations of PBDEs via their diet by daily injecting the commercial technical mixture, DE-71, into their day-old frozen-thawed cockerels. Non-destructive sampling (plasma samples) was employed.

Adult kestrels, prior to egg laying in the breeding seasons of 2005 and 2006, were assessed for effects on their retinol levels. The subsequent focus was on specific effects of embryonic or *in ovo* exposure to PBDEs. The young exposed *in ovo* were assessed for effects on their thyroid hormones and retinol levels while nestlings. Together, these results will provide an important understanding of how exposure to environmentally relevant concentrations of PBDEs, one class of BFR contaminants, commonly found in the environment, affects the reproduction and growth of birds.

Table 1. Classification of the main BDE congeners found in the three PBDE commercial mixtures.

Major BDE congeners in the three PBDE commercial mixtures	Congener classification						
	TetraBDE	PentaBDE	HexaBDE	HeptaBDE	OctaBDE	NonaBDE	DecaBDE
DE-71							
BDE-47	X						
BDE-99		X					
BDE-100		X					
BDE-153			X				
DE-79							
BDE-183				X			
BDE-196					X		
BDE-197					X		
BDE-207						X	
Saytex 102E							
BDE-209							X

CONNECTING STATEMENT

In the first chapter, a literature review on polybrominated diphenyl ethers (PBDEs) was presented, which included their use, chemical structure and properties, release and occurrence in the environment, and their effects on wildlife. There has been little investigation into the effects of PBDEs on vitamin A (retinol) levels. Retinol is important for a number of functions, which include reproduction, appropriate embryonic development and growth. With dietary exposure to PBDEs, American kestrels (*Falco sparverius*) demonstrated reduced reproductive success as well as altered growth in the nestlings. The investigation of retinol in these exposed birds in chapter 2 may enable a physiological understanding behind the observed changes in reproduction and development.

CHAPTER 2 - The effects of a dietary or *in ovo* exposure to the pentaBDE mixture, DE-71, on retinol levels of captive American kestrels (*Falco sparverius*): a possible explanation for changes in reproduction and growth

Abstract

Polybrominated diphenyl ethers (PBDEs) are ubiquitous environmental pollutants that comprise one class of brominated flame retardants commonly found in office and household items. Their lipophilic nature and ability to bioaccumulate are causes for concern, especially for those species at the top of the food chain such as raptors. In this study, plasma retinol levels of a small falcon, the American kestrel, were assessed during the breeding season after three weeks of dietary exposure to DE-71, a commercial mixture of PBDEs. As well, plasma retinol levels in their young were assessed following their *in ovo* exposure to DE-71. The experimental design consisted of three exposure groups (daily dietary exposure): control (vehicle only), low (0.3 ppm DE-71), and high (1.6 ppm DE-71). Maternal retinol levels were reduced in the low-exposure group and were associated with fewer hatchlings and increasing *in ovo* BDE-17 concentrations. The low *in ovo* exposed nestlings' retinol levels were also reduced, with a positive association with the birds' body mass at 25 days of age. This study indicates that maternal retinol levels in American kestrels prior to the breeding season, as well as their nestlings at 25 days of age, are responsive to PBDE exposure, particularly for lower PBDE concentrations that are found in wild birds. Further research is needed to understand the differential response among treatments.

Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardants commonly found in industrial, office and household items such as furniture, electronic appliances, carpets and clothing (Rahman et al. 2001). The pentaBDE mixture used in this study, DE-71, is added to the products and is not chemically bound. This facilitates its release into the environment, which occurs slowly over the lifespan of a product (McDonald 2002). PBDEs can also enter the environment through effluents and flue gases from factories, and can indirectly enter the food chain via diet (Watanabe and Sakai 2003). PBDEs are

comparable chemically and physically to PCBs, having similar properties of stability and lipophilicity (Rahman et al. 2001). These characteristics result in their persistence and bioaccumulation in biota.

PBDEs are ubiquitous and have been found to be increasing in the environment at an exponential rate (de Wit 2002), although some PBDEs and other brominated flame retardants are now stable or declining in some geographical locations (Gauthier et al. 2007; Chen et al. 2008). For example, herring gull (*Larus argentatus*) eggs from the Great Lakes region had increasing concentrations of PBDEs from 1981 to 2000 (Norstrom et al. 2002). While there have been recent declines of some PBDE congeners in the eggs of herring gulls from that region when comparing levels from 2000 to 2004 (Gauthier et al. 2007), more recently there has been an increase in other PBDE congeners (Gauthier et al. 2008). This overall global pattern is most likely a response to three events: Europe's ban of penta- and octaBDEs in 2004 (Costa and Giordano 2007), the voluntary discontinuation of penta- and octaBDE production by their U.S. producer in 2004 (Renner 2004), and their subsequent ban in several states (Costa and Giordano 2007) and in Canada (Canada Gazette 2006) in 2006. The presence of PBDEs in the environment remains a concern especially for those at the top of the food chain, such as raptors, where bioaccumulation of PBDEs occurs most acutely.

The possible impact of PBDEs on avian species is poorly understood. PBDE congener concentrations have been found in common cormorants (*Phalacrocorax carbo*) in England, Wales (Law et al. 2002), and Japan (Watanabe et al. 2004), in glaucous gulls (*Larus hyperboreus*) in Norway, and in guillemots (*Cepphus* sp.) in several environments (Sinkkonen et al. 2004). They have also been detected in the eggs of peregrine falcons (*Falco peregrinus*) in Sweden (Lindberg et al. 2004, Johansson et al. 2009) and other raptorial birds in Norway, i.e., merlins (*F. columbarius*) and golden eagles (*Aquila chrysaetos*): Herkze et al. 2005. Fairly recently, the highest levels of PBDEs reported were found in the common kestrel (*F. tinnunculus*) in China (Chen et al. 2007). The implications of these high concentrations of PBDEs in avian tissue are not fully understood as there are few studies detailing their physiological effects. However,

validated tests such as this study will be useful for filling in at least some knowledge gaps.

Retinol concentrations are critical for a number of important functions such as reproduction and appropriate embryonic and nestling growth (Ganguly 1989, Zile 1998). Similar environmental contaminants, such as PCBs, have been found to affect vitamin A (retinol) levels in a number of species (see review: Rolland 2000). There have, however, been few controlled PBDE experiments involving physiological impacts upon avian species. When exposed to PBDE congeners during embryonic and nestling development, American kestrel (*F. sparverius*) fledglings demonstrated marginal effects in plasma and hepatic vitamin A (retinol) levels (Fernie et al. 2005). A possible disruption of retinol by the experimental exposure of American kestrels to PBDEs, i.e., DE-71, could partially explain the changes in the kestrels' growth (Fernie et al. 2006) and reproductive success (Fernie et al. 2009), the increased sensitivity of kestrel embryos to PBDEs compared to chickens (*Gallus gallus*) and mallards (*Anas platyrhynchos*) (McKernan et al. 2009), and finally, the reduction in brood size associated with PBDE-exposure found in peregrine falcons in Sweden (Johansson et al. 2009).

As part of a larger research study (Fernie et al. 2008, 2009), this study examines the effect of a dietary exposure of the pentaBDE mixture, DE-71, on plasma retinol concentrations in adult American kestrels prior to the breeding season, as well as the effect of *in ovo* DE-71 exposure on retinol levels in their nestlings.

Methods

Study site and details

This study was conducted at the Avian Science and Conservation Centre (ASCC; McGill University, Montreal). During the summers of 2005 and 2006, 62 American kestrels of known age and pedigree were paired for breeding each year (2005: April 21, 2006: April 7). Randomly selected pairs were assigned to either a high (1.6 ppm DE-71; 2005: n = 11, 2006: n = 10), low (0.3 ppm DE-71; 2005: n = 10, 2006: n = 10), or control (vehicle only (safflower oil); 2005: n = 10, 2006: n = 11) exposure group. The birds from

2005 were paired with different birds within the same treatment in 2006. The resulting high- and low- exposure concentrations were based on environmentally relevant levels of PBDEs found in herring gull eggs collected in the Great Lakes Basin (Norstrom et al. 2002, Gauthier et al. 2007). The *in ovo* levels of both exposure groups are also equivalent to maximal levels found in American kestrel eggs in southern Ontario (P. Martin, Environment Canada, upubl. data) and peregrine falcon eggs in central and eastern Canada (K. Fernie, Environment Canada, upubl. data), and similar to the concentrations reported in peregrine eggs in the northeastern U.S. (Chen et al. 2008).

The complete chemical preparation for exposure can be found elsewhere (Fernie et al. 2008). Briefly, the three exposure mixtures were prepared using 250 mL of the same safflower oil whereby DE-71 was added (high: 0.1645 g, low: 0.0351 g, control: safflower oil only) to prepare the final mixture concentrations (high: 0.658 μg/μL, low: 0.140 μg/μL). Using three repeatable injecting syringes, i.e., one for each exposure group, the appropriate amount of DE-71 (high: 0.65 μg/μL, low: 0.12 μg/μL) or 100 μL safflower oil for the controls were injected intracranially into day-old frozen-thawed cockerels, which were provided on an *ad libitum* daily diet.

Dietary exposure to the pentaBDE mixture, DE-71, or the control vehicle began approximately three weeks before pairing (2005: March 26; 2006: March 17) and continued throughout the courtship, laying and incubation period (approximately 75 d) until the first chick (would have) hatched. Each breeding pair was housed in a breeding pen (1.0 m x 2.4 m x 2.4 m: W x L x H) containing a nest box (0.3 m x 0.3 m x 0.4 m: W x L x H), two rope perches, a one-way glass observation window (0.1 m x 0.3 m x 0.01 m: W x L x H) and a food platform (0.15 m x 0.15 m x 0.01 m: W x L x H). Their care and all experimental protocols were conducted according to the guidelines of the Canadian Council on Animal Care (Olfert et al. 1993) and approved by the McGill University Animal Care Committee (Appendix A).

Sampling procedures

Immediately prior to the pairing of adult males and females, birds were weighed and whole blood samples (1.0 mL) were collected using a 27 gauge, ½" needle (2005 and 2006: high n = 21(♀), 19(♂); low n = 20(♀), 17(♂); control n = 18(♀), 18(♂)). Body mass and blood samples (0.5 mL) were also collected using a 27 gauge, ½" needle from nestlings at 25 d of age in 2005 (high n = 7(♀), 10(♂); low n = 7(♀), 16(♂); control n = 14(♀), 10(♂)). The plasma from all blood samples was separated by centrifugation and stored at -80°C until completion of the retinol analysis.

Plasma vitamin A (retinol)

The plasma samples were extracted and analyzed by high performance liquid chromatography (HPLC) in isocratic condition (100% methanol; 1 mL/min) where 50 µL of plasma was used. The retinol concentration was calculated by the internal standard method where retinyl acetate was used as the internal standard. Following the addition of retinyl acetate to the 50 µL aliquot of plasma, the retinol-protein complex was dissociated with acetonitrile and the retinol was extracted by successive volumes of hexane. Centrifugation was used to separate the organic and aqueous phases and the organic phases were then combined and evaporated to dryness with nitrogen. The residues were then re-dissolved in methanol, filtered and analyzed. The samples were injected on the Waters Alliance 2795 XE HPLC equipped with a photodiode array (PDA) detector, Waters 2996 PDA, set at 325 nm and the retinol concentrations were determined. The analytical methods used for measuring retinol are the same as used by Fernie et al. (2005).

Reproductive measures

As per Fernie et al. (2009), nest boxes were checked daily to determine clutch sizes, lay and hatching dates. Other reproductive endpoints collected included the fertility of the eggs at mid-incubation, the number of hatchlings per pair, and the proportion of fertile eggs that hatched. The morphometric size of each nestling was also determined on the day of hatching and subsequently every 5 days (according to the oldest hatchling) until 30 days of age. In the American kestrel, hatching of most nestlings occurs within 24

h of the first hatchling emerging, and occasionally within 48 h. The morphometric parameters collected included the body mass (recorded to a tenth of a gram using a Sartorius Model PT600 scale) and tibiotarsal (tarsus) bone length to a hundredth of a millimeter using a digital caliper.

Chemical analysis

The first egg of each clutch was collected and used for determining PBDE concentrations as per Gauthier et al. (2007). A detailed description of the chemical analysis and the data for PBDEs and hexabromocyclododecan (HBCD) in the present kestrel eggs can be found elsewhere (Fernie et al. 2008, 2009). Adult retinol samples were obtained in 2005 and 2006 and since the concentrations in the eggs were similar for 2005 and 2006, the data were combined. Briefly, mean ΣPBDE concentrations found in these eggs were 3.01 ± 0.46 ng/g ww for the control group, 288.60 ± 33.35 ng/g ww for the low-exposure group, and 1130.59 ± 95.34 ng/g ww for the high-exposure group. The congener profile detected in the eggs was as follows, in decreasing order of their contribution to the ∑PBDE *in ovo* burden: BDE-99 (35%), -153 (18.5%), -100 (10%), -154 (10%), -47 (7.5%), -138 (6.5%), -183 (4.5%), -209 (3%), -190 (2.5%), HBCD (1%) and the remaining 1.5% was made up of BDE-85, -49, -66, -17, -28. HBCD was only analyzed in the eggs from 2006 and was not expected to be present in the egg samples. It was found to be an exposure artifact during the dosing of the birds (Fernie et al. 2008, 2009).

The nestling retinol levels were only examined in 2005. The mean ΣPBDE concentrations found in the eggs from only 2005 were 2.88 ± 0.26 ng/g ww for the control group, 290.66 ± 48.39 ng/g ww for the low-exposure group, and 1111.44 ± 160.38 ng/g ww for the high-exposure group. The congener profile detected in the eggs in 2005 was as follows, in decreasing order of their contribution to the ∑PBDE *in ovo* burden: BDE-99 (39.5%), -153 (18.5%), -100 (11%), -183 (9.5%), -154 (9%), -47 (8.5%), -138 (2%), -85 (1.5%), and the remaining 1% was made up of BDE-49, -209, -28, -66, -17 in decreasing order. This profile is similar to the combined 2005 and 2006 congener profile with some minor exceptions, e.g., BDE-183, -209.

Statistical analysis

All statistical analyses were performed using SAS 9.2 (SAS Institute Inc. 2002-2008). All data were normally distributed or log-transformed accordingly. To determine differences in the adult retinol levels among exposure groups, one-way analysis of variance (ANOVA) was used with *post-hoc* pairwise comparisons of the least-squared means (LSM). Pearson's correlations were also performed to determine possible associations for the adult retinol levels with their body mass, reproductive endpoints (clutch size, lay dates, the fertility of the eggs at mid-incubation, the proportion of fertile eggs that hatched, and the number of hatchlings per pair), and *in ovo* BDE concentrations. Differences relating to the sex of the nestling birds were identified prior to further statistical analysis. The nest was used as the nested factor to control for possible differences in parental care as any difference in food provisioning behaviour by the parents will affect retinol levels; the precursor to retinol is acquired through a diet of animal origin (Ganguly 1989). To determine differences among the exposure groups in nestling retinol concentrations, nested one-way ANOVAs were used with nest as the nested factor to control for possible differences in sibling or parental care qualities; the nested ANOVAs were followed by *post-hoc* pairwise comparisons of the LSM. Pearson's correlations were performed for nestling concentrations of retinol with *in ovo* BDE congener concentrations, as well as the nestling morphometric size measurements. Statistical significance was determined at $p < 0.05$, and means ± standard errors (SEMs) are presented.

Results

Retinol concentrations were significantly different among adult females ($F_{2,56}$ = 3.87, p = 0.027, Fig. 1) but not for the adult males ($F_{2,50}$ = 0.540, p = 0.584). The low-exposure adult females had significantly lower retinol concentrations than the control adult females (LSM p = 0.045), but interestingly, the high-exposure birds did not follow this pattern. There was a marginal negative correlation between the adult female retinol levels and the *in ovo* BDE-17 concentrations (n = 48, r = -0.262, p = 0.072), and for the

low-exposure females, their numbers of hatchlings increased with increasing retinol concentrations (n = 18, r = 0.516, p = 0.028, Fig. 2).

There was not a significant difference between the sexes when controlling for parental care for the nestling retinol levels ($F_{28,35}$ = 1.50, p = 0.270) so the sexes were combined. There was a statistically significant difference among exposure groups for the nestling retinol concentrations ($F_{18,48}$ = 1.91, p = 0.042, Fig. 1) with the low *in ovo* exposed nestlings having significantly lower retinol levels than the controls (LSM p = 0.026). The retinol levels of the nestlings were also significantly and positively correlated with their body mass at the time of sampling (25 d; n = 60, r = 0.271, p = 0.030, Fig. 3).

Discussion

The dietary exposure to the pentaBDE mixture, DE-71, was found to reduce maternal and nestling retinol levels in the low exposure group and the low *in ovo* exposure group, respectively. The maternal retinol levels were associated with *in ovo* concentrations of BDE-17, and for the low-exposed females, the number of hatchlings. The nestlings' retinol levels were positively associated with their body mass at 25 days of age.

Several studies have found a reduction in retinol levels following dietary exposure to contaminants, particularly for PCBs, with few studies focusing on PBDEs and effects on retinol levels in birds. Exposure by diet to PCBs caused a reduction in hepatic retinol levels in ring doves (*Streptopelia risoria*), although the serum retinol levels were not significantly different in the exposed female ring doves (Spear et al. 1989). The study by Spear et al. (1989), however, consisted of only one intraperitoneal injection of a much higher dose of PCBs (40 μg/g) one week before pairing compared to the multi-day dietary exposure of the kestrels to the DE-71 technical mixture in this study. In the female ring doves, the lack of response to the PCB exposure in terms of the female plasma retinol levels (Spear et al. 1989) contrasts with the significant response in plasma retinol levels of the adult PBDE-exposed female kestrels. A 14 d oral exposure in adult female rats and mice to penta- and tetra-BDEs resulted in reduced hepatic vitamin A levels (retinol and

retinyl esters) for the pentaBDE exposure only (Bromkal 70-5: 18 and 36 mg/kg body weight per day and DE-47: 18 mg/kg body weight per day, respectively, totalling 250 or 500 mg/kg body weight; Hallgren et al. 2001), although concentrations fed to these laboratory animals were again much greater than in the present study.

In this study, the adult retinol levels were assessed on the day the males and females were paired, which was three weeks after dietary exposure to the DE-71 mixture began and at the beginning of the reproductive period. Egg laying was delayed in the PBDE-exposed kestrels, beginning, after pairing, on average 22.72 ± 3.23 d for the low exposure and 28.39 ± 3.71 d for the high exposure compared to 18.68 ± 2.06 d for the controls (Fernie et al. 2009). Changes in plasma retinol concentrations between treatment groups occurred in the adult females only, but not in the adult males or nestlings. Retinol is only released when needed, and is critical for egg laying. Depressed retinol levels in birds are associated with reduced reproductive capacity (Spear et al. 1990, Fox 1993). The environmental concentrations to which the adult kestrels were exposed were sufficient to reduce maternal retinol levels after only three weeks of dietary exposure. Changes in retinol concentrations were unlikely to occur in the adult males, since their retinol demands, and hence the release of retinol during this phase, were likely relatively minimal at the time of sampling. In contrast, female adult birds would have had high requirements for retinol with the approach of egg laying and the occurrence of follicular development and maturation during this three week period of exposure to DE-71.

As discussed above, retinol is critical for successful reproduction (Ganguly 1989). In extreme cases of vitamin A deficiency, a rapid reduction in egg laying has been observed (Thompson 1969) and early embryonic death can occur (Zile 1998). A reduction in vitamin A levels in the PBDE-exposed females prior to egg laying may have contributed to the reduced reproductive success, which included reduced egg fertility, observed in these exposed birds (Fernie et al. 2009). A contributing factor to the reduced retinol levels seen in the low-exposure females could be their reduced food consumption during the courtship period (Fernie et al. 2008), as the precursor to retinol is acquired through a diet of animal origin (Ganguly 1989). In addition, the *in ovo* concentrations of

BDE-17 were also marginally associated with reduced maternal plasma retinol levels. This marginal correlation between retinol and *in ovo* BDE-17 concentrations was surprising as this congener was found in one of the lowest concentrations in the eggs in this study, as well as in herring gull eggs from the Great Lakes region (Norstrom et al. 2002), and yet was also associated with the delay in egg laying and the reduced thickness and mass of the eggshell (Fernie et al. 2009). Together, these various associations, including those with the affected retinol concentrations, suggest that low concentrations of some PBDE congeners are sufficient to elicit biological responses.

The pentabrominated flame retardants affecting retinol levels in breeding females may also have implications in terms of embryonic exposure. The PBDE-exposed female kestrels, particularly in the high-exposure group, laid smaller eggs that lost more weight during embryonic development and had poorer hatching success (Fernie et al. 2009). The appropriate amount of retinol received by the embryo is derived from the female through deposition in the yolk, followed by the yolk sac membrane and into the liver of the embryo (Joshi et al. 1973, Surai et al. 2001). Retinol is important for embryonic development (Zile 1998). The vitamin A levels assessed in chicken embryos have been found to decrease throughout the incubation phase and the amounts derived maternally are used for embryonic development (Joshi et al. 1973). There was a sufficient amount remaining for 7 d after hatching for growth until the nestlings were able to meet their own vitamin A requirements through the consumption of an external source of vitamin A (Joshi et al. 1973). If vitamin A received by the embryo from the maternal source is reduced, then it may affect the embryo's development, contributing to the reduced hatching and overall reproductive success seen in these PBDE-exposed birds (Fernie et al. 2009). Furthermore, McKernan et al. (2009) reported a reduction in pipping and hatching success of kestrel embryos following their embryonic exposure to DE-71. This finding is supported by the current study's correlation where the reduced low-exposure maternal retinol levels produced fewer hatchlings (Fig. 2). In addition, the smaller eggs seen in these exposed birds (Fernie et al. 2009) could also be an indication of poorer egg quality, including their nutrient content. Lesser scaup (*Aythya affinis*) ducklings from

larger eggs were larger at hatching and had better survival, reportedly due to an allocation of more nutrients in the eggs (Dawson and Clark 1996).

Although retinol levels in the embryo were not assessed, the nestling retinol levels from this study were also reduced by the low *in ovo* PBDE exposure at 25 days of age. Several studies have found reduced nestling retinol levels associated with PCB and PBDE *in ovo* exposure. For example, PCB exposure was negatively associated with plasma retinol levels in European shag (*P. aristotelis*) hatchlings (Murvoll et al. 2006). Similarly, black guillemot (*Cepphus grylle*) nestlings had reduced retinol levels in association with PCB exposure (Kuzyk et al. 2003). One *in ovo* injection (1500 ng/g ∑PBDEs) coupled with ongoing developmental exposure (mean 15.6 ± 0.3 ng/g/bird/day) of 29-day old nestling American kestrels to PBDEs marginally reduced plasma and hepatic retinol and retinyl palmitate levels as juveniles (36 days old; Fernie et al. 2005) at exposure concentrations that were higher (1500 ng/g ww) than the high-exposure concentrations used in the current study. The difference in their exposure routes, embryonic only in this study versus embryonic and developmental, as well as the lack of a low-exposure group in Fernie et al. (2005) and the longer exposure time to the higher PBDE concentrations in the earlier study (Fernie et al. 2005), may explain the differences in the observed changes to the retinol levels, i.e., the reduced plasma retinol levels in the low *in ovo* exposed nestlings in this study compared to a marginal reduction in retinol of the kestrel juveniles (Fernie et al. 2005). There was one yolk injection study involving an *in ovo* exposure to BDE-99 (0.1, 1, or 10 ng/g ww) in domestic ducks where there was no effect on hepatic or plasma retinol levels (Murvoll et al. 2005). This lack of a response may be due to ducks being less sensitive than kestrels to PBDE exposure, as was seen when comparing PBDE-exposed kestrel and mallard embryos where the kestrel embryos experienced reduced pipping and hatching success (McKernan et al. 2009).

The *in ovo* exposed nestlings may have received a reduced amount of maternally-derived retinol due to disruption from the PBDE exposure. As stated previously, the maternally derived vitamin A stores in the yolk are used by nestlings for several days after hatching (Joshi et al. 1973) until they begin using external retinol sources from their

diet. In this study, the nestlings received a cockerel diet rich in retinols and carotenoids (Karadas et al. 2005). The plasma retinol levels in this study were assessed at 25 days of age, when the nestlings had likely gained enough maternally-derived retinol post-hatch to ensure their survival and to begin receiving external dietary retinol sources. The *ad libitum* diet of cockerels that these captive kestrels are fed does not, however, appear to be sufficient for possible retinol compensation by the nestlings in the low exposure group. This may be accounted for in the reduced parental behaviours, i.e., reduced food provisioning behaviour, observed in adult males of the low-exposure group who spent a marginally reduced time in their nest boxes compared to the controls (S. Marteinson, unpublished data). This assessment is supported by a DE-71 exposure (18 mg/kg daily) study whereupon pregnant rats were exposed orally from gestation day 6 through lactation day 18 in addition to either a sufficient or marginal vitamin A diet (Ellis-Hutchings et al. 2006). The young rats in that study had lower plasma retinol levels only with the marginal vitamin A diet (Ellis-Hutchings et al. 2006).

A reduced amount of retinol in the nestlings initially after hatch due to a reduced amount of retinol received maternally could have been detrimental to the initial growth of the kestrel chicks as vitamin A is also required for growth (Ganguly 1989). Indeed, female nestlings at 5 days of age from the low-exposure group were lighter and had shorter tarsal bones, and their maximal growth period was also delayed at 5 days of age, compared to the female control nestlings (K.J. Fernie, Environment Canada, upubl. data). The potentially reduced maternally-derived retinol in these kestrel nestlings may, therefore, have contributed to the alterations of their early growth. Depressed retinol levels in birds are associated with delayed growth and developmental deformities (Spear et al. 1990, Fox 1993). Pre-fledging herring gulls and Caspian terns (*Sterna caspia*) had reduced retinol levels that were correlated with increasing PCB exposure and also demonstrated a positive correlation between retinol levels and mass (Grasman et al. 1996). The importance of retinol for growth is further corroborated in our study by the positive correlation between nestling retinol levels and their body mass at 25 days of age (Fig. 3), and by the finding that the low-exposure male and female nestlings who experienced reduced retinol levels were significantly lighter in body mass than their

respective controls at 25 days of age (K.J. Fernie, Environment Canada, unpubl. data). The statistically reduced retinol levels in the low *in ovo* exposed nestlings may be representing their compensation for their reduced body mass in that they have expended more retinol at this time (25 d) to achieve their maximal size. Immediately prior to the sampling at 25 days of age was the completion of the kestrels' maximal growth rates (5 to 20 d) and asymptotic size (20 to 22 d). The nestlings would therefore have had high requirements for retinol immediately prior to sampling (25 d). With PBDE exposure disrupting retinol levels in the low *in ovo* exposed nestlings, the effects will be and are represented in their reduced size.

The mechanism by which retinol is disrupted by PBDE exposure has been investigated to some extent (Spear et al. 1986, 1988; Zhou et al. 2001). Interruption of retinol is most likely a result of an inability to maintain or replace the reducing available stores or by increasing retinol clearance from the body. As proposed by Brouwer et al. (1985), a reduction in the synthesis or secretion of the transport proteins of retinol due to exposure could be altering the level of transport of retinol within the body. The increased clearance of retinol from an organism could be due to an increase in enzyme activity, due to exposure, metabolizing the retinol (Spear et al. 1986, 1988; Zhou et al. 2001). In addition, retinol and thyroid hormones are transported in blood on the protein transthyretin (TTR), and some of the PBDE congeners and their metabolites have a stronger binding affinity for TTR than thyroid hormones (Ucan-Marin et al. 2008) and potentially retinol, resulting in perturbation of these parameters. Identification of the mechanism behind the changes in retinol of the kestrels is, however, beyond the scope of the current study but does require further investigation.

In conclusion, plasma retinol levels are sensitive to environmentally relevant dietary PBDE exposure in pre-laying female American kestrels and the plasma retinol levels of their young exposed *in ovo* to PBDEs. The reduced maternal retinol levels, and the effects of the *in ovo* PBDE exposure on embryonic development and nestlings, may have contributed to the reduced reproductive success and growth, respectively, of these birds. The reduced maternal and nestling plasma retinol levels found in the present study

are further evidence that dietary exposure to environmentally relevant levels of PBDEs disrupts the retinol system, especially during the critical reproductive and growth phases. This also indicates that the avian retinol system, when under high physiological demand, is sensitive to disruption at exposure levels that are currently found in avian biota. The changes and associations observed in the retinol levels in the kestrels as a function of their PBDE exposure in this study, may be exacerbated in wild birds, which, in contrast to the captive kestrels, are continuously exposed to contaminants and also consume a natural diet that is not enriched with retinol and carotenoids.

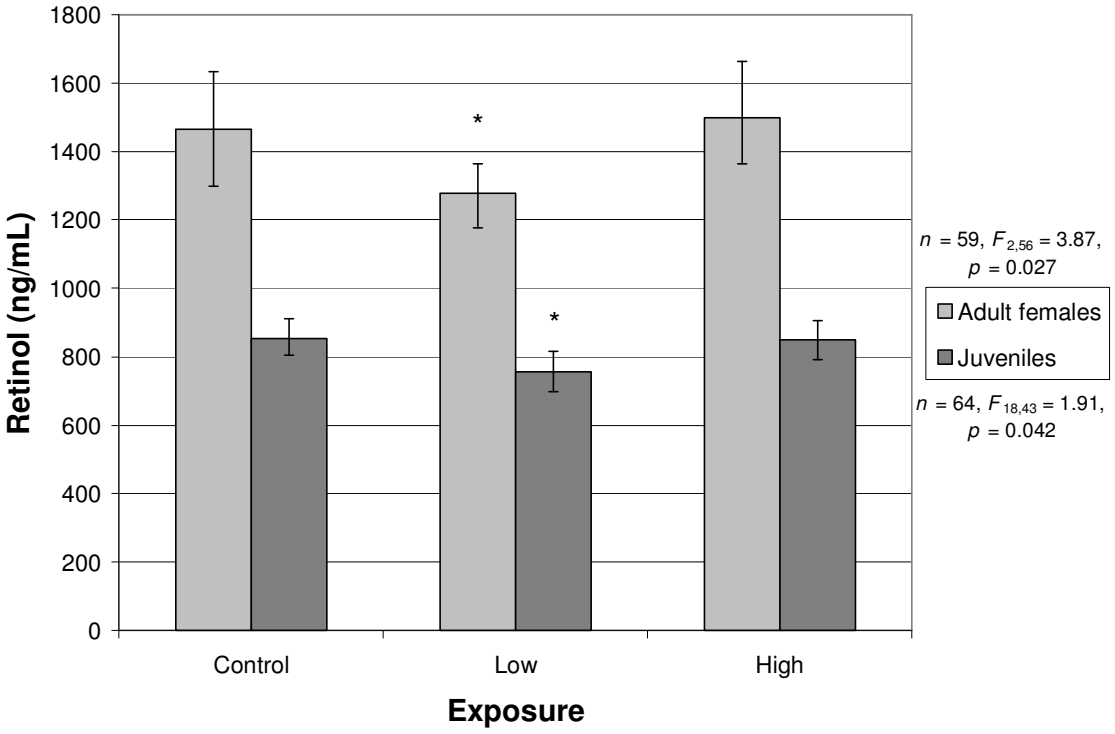

Fig. 1. Retinol levels of American kestrel (*Falco sparverius*) females and nestlings exposed to PBDEs via diet and *in ovo*, respectively. *LSM significant difference from the controls ($p < 0.05$).

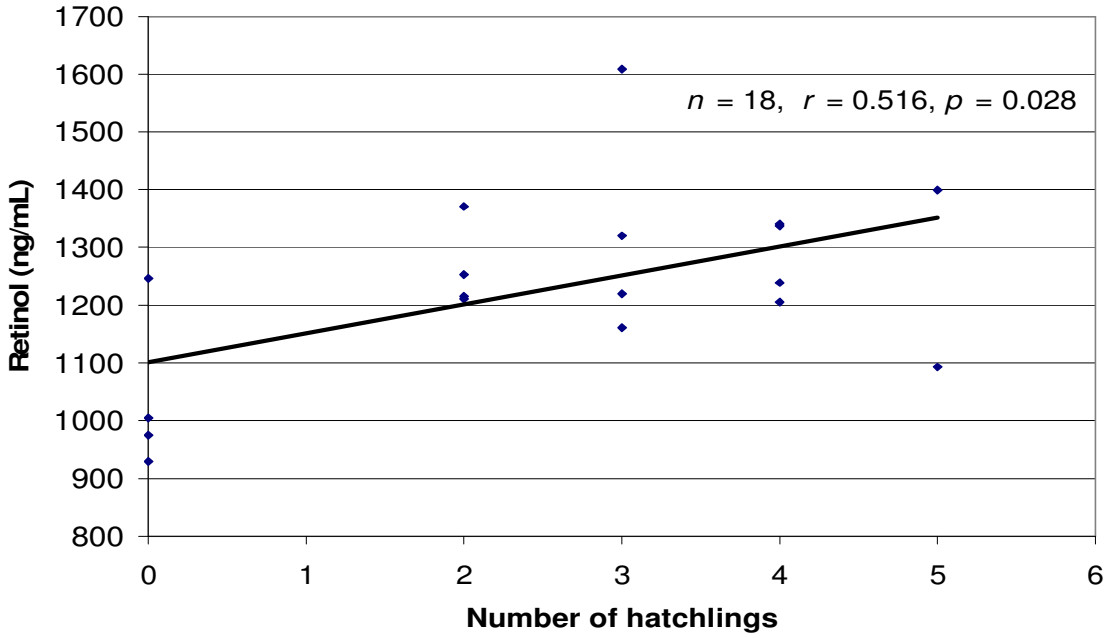

Fig. 2. Pearson correlation analysis for the low dietary PBDE exposure group: plasma retinol concentrations of the adult female American kestrels and their number of hatchlings per pair.

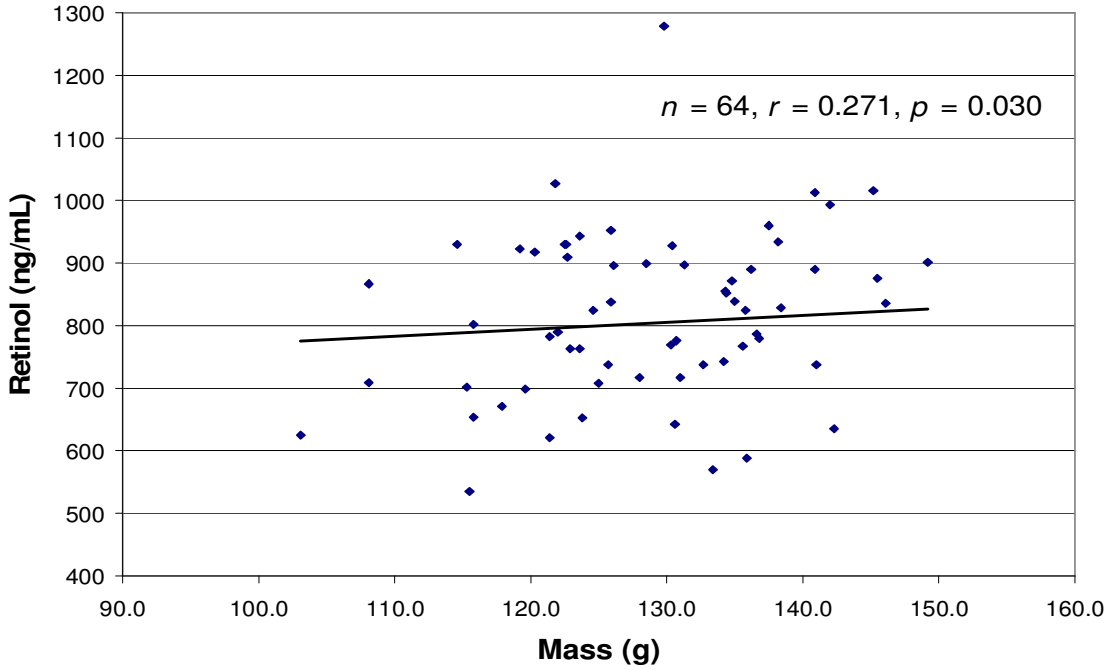

Fig. 4. Pearson correlation analysis: plasma retinol concentrations of PBDE *in ovo* exposed nestling American kestrels and their body mass at 25 days of age.

CONNECTING STATEMENT

The second chapter assessed the retinol levels in adults following their dietary exposure to PBDEs and in their *in ovo* exposed nestlings. Maternal retinol levels were altered by the environmentally relevant PBDE exposures, and their reduced levels were associated with fewer hatchlings and *in ovo* BDE-17 concentrations. This may partly explain the reduced reproductive success seen in these birds. The retinol levels in their *in ovo* exposed young were not affected by the PBDE exposure and therefore the underlying physiology of their altered growth remains unclear. Chapter 3 will assess the thyroid hormones, total triiodothyronine (TT_3) and thyroxine (TT_4), the function of the thyroid (TT_3:TT_4 ratio), and the response of the thyroid hormones following an injection of the thyroid-stimulating hormone (TSH). The thyroid system is important for a number of functions, which include appropriate development and growth. These *in ovo* exposed nestlings experienced altered growth and examining their thyroid system may provide an underlying endocrine explanation.

CHAPTER 3 - The effects of an *in ovo* exposure to the pentaBDE mixture, DE-71, on thyroid hormones of nestling captive American kestrels (*Falco sparverius*)

Abstract

Polybrominated diphenyl ethers (PBDEs) are a type of flame retardant used to reduce the spread of fires. Found worldwide in both the environment and biota, their lipophilic and persistent properties, similar to polychlorinated biphenyls (PCBs), and their occurrence in wildlife are causes for concern. The current study involved the *in ovo* exposure of American kestrels to the pentaBDE mixture, DE-71, during the embryonic development stage. The experimental design consisted of three exposure groups with *in ovo* levels of DE-71 of 1145.48 ± 123.41 ng/g ww (high exposure), 286.78 ± 48.79 ng/g ww (low exposure), and 3.14 ± 0.86 ng/g ww (control). These levels are consistent with environmentally relevant levels found in wild peregrine falcons (*Falco peregrinus*) and herring gulls (*Larus argentatus*). Thyroid levels were assessed in the nestlings at several ages (17, 19, and 20 days old) as was their response to an injection of the thyroid-stimulating hormone (TSH; 17 and 19 days old). PBDE exposure was found to affect plasma concentrations of total triiodothyronine (TT_3) and thyroxine (TT_4), as well as the TT_4:TT_3 ratio at 20 days of age only. The low-exposure nestlings had increased TT_3 levels but suppressed TT_4 levels and TT_4:TT_3 ratio, particularly for the female nestlings. The high-exposure nestlings had reduced TT_3 levels at 20 days of age and a reduced TT_4 response following TSH injection. These exposure-affected thyroid parameters were associated with several *in ovo* BDE congener concentrations. TT_3 levels were particularly associated with body mass and TT_4 concentrations and responses were especially associated with body mass and feather growth of the nestlings. The thyroid system is therefore sensitive to disruption by *in ovo* PBDE exposure.

Introduction

Polybrominated diphenyl ethers (PBDEs), a type of brominated flame retardant, are used in common industrial, household and office items such as furniture, electrical appliances, paints, and textiles (Rahman et al. 2001). They also have chemical and physical properties similar to PCBs whereby they are lipophilic and persistent in the

environment (Rahman et al. 2001). Because these properties allow for their bioaccumulation in biota (Law et al. 2003), PBDEs are found worldwide in both biota and the environment (Birnbaum and Staskal 2004). The additive nature of this flame retardant, i.e., it is added and not chemically bound to the products, facilitates its eventual leaching from the products into the environment and the food chain (McDonald 2002).

The pentaBDE mixture, DE-71, used in this study had a high market demand in North America compared to Asia and Europe (Rahman et al. 2001) until the voluntary cessation of production by the U.S. manufacturer in 2004 (Renner 2004) and subsequent banning in several states (Costa and Giordano 2007) and Canada (Canada Gazette 2006) in 2006. This does not, however, prevent the continued release of PBDEs into the environment from products currently in use. Found in a number of ecosystems as remote as the Arctic (de Wit et al. 2006), PBDEs have been exponentially increasing in the environment and biota (de Wit 2002). For example, PBDE levels in herring gull (*Larus argentatus*) eggs of the Great Lakes Basin had been exponentially increasing from 1981-2000 (Norstrom et al. 2002), as have those of peregrine falcons (*Falco peregrinus*) in Greenland with a 10% increase per year (1986-2003; Vorkamp et al. 2005), double-breasted cormorants (*Phalacrocorax auritus*) and great blue herons (*Ardea herodias*) in British Columbia with a doubling time of 5.7 years (1979-2002: Elliot et al. 2005). PBDEs have also been detected in the eggs of a number of other predatory birds, i.e., merlins (*F. columbarius*), golden eagles (*Aquila chrysaetos*) and goshawks (*Accipiter gentilis*) (Herzke et al. 2005), and little owls (*Athene noctua*; Jaspers et al. 2005). Some of the highest levels of PBDEs have been found fairly recently in the common kestrel (*F. tinnunculus*; Chen et al. 2007). The chemical properties of PBDEs and their occurrence in many species globally are cause for concern for wildlife species, especially those at the top of the food chain, such as raptors, where bioaccumulation of PBDEs occurs most acutely.

Few studies have detailed the physiological effects of PBDEs in avian species, particularly those at high risk for bioaccumulation like raptors. However, PBDEs have been found to contribute to a number of reproductive and physiological disruptions.

American kestrel (*F. sparverius*) embryos were particularly sensitive to an egg injection of DE-71 compared to ducks (*Anas platyrhynchos*) and chickens (*Gallus gallus*), i.e., only the kestrels demonstrated decreased pipping and hatching (McKernan et al. 2009). Exposure to PBDEs also caused reductions in the reproductive success of American kestrels, e.g., reduced fertility and hatching success (Fernie et al. 2009), and was negatively associated with reproductive performance in peregrine falcons, i.e., reduced brood size (Johannson et al. 2009), and ospreys (*Pandion haliaetus*; Henny et al. 2009). Growth of nestling kestrels has also been altered by PBDE exposure (Fernie et al. 2006, K.J. Fernie Environment Canada, upubl. data). Finally, hormonal disruption, particularly of thyroid hormones, has been shown in rodents (Zhou et al. 2001, 2002; Hallgren et al. 2001), kestrels (Fernie et al. 2005), and ranch mink (*Mustela vison*; Zhang et al. 2009).

This study, which is part of a larger research study (Fernie et al. 2008, 2009), consists of a controlled experiment whereby American kestrels were exposed by diet prior to and during the breeding season to environmentally relevant levels of PBDEs. These levels are consistent with concentrations found in herring gull eggs from the Great Lakes Basin (Norstrom et al. 2002, Gauthier et al. 2007). Thus, their young were also exposed to PBDEs *in ovo* and we sought to identify any underlying thyroid hormone disruption to those nestlings. Thyroid hormones are critical for normal nestling growth (McNabb 2000) and any changes in their levels due to exposure to PBDEs could lead to alterations in their growth patterns. This study will also contribute to a better understanding of the physiological disruption caused by *in ovo* PBDE exposure in terms of the thyroid hormones and the functioning of the thyroid gland.

Methods

Study site and details

On April 7, 2006 at the Avian Science and Conservation Centre (McGill University, Montreal), 62 birds of known age and pedigree were paired. The 31 pairs were randomly assigned to three exposure groups: high (1.6 ppm DE-71, $n = 10$), low (0.3 ppm DE-71, $n = 10$), or control (vehicle only (safflower oil), $n = 11$). These high and low levels are environmentally relevant and consistent with those found in the eggs of

herring gulls of the Great Lakes Basin in 2000 or 2004 (Norstrom et al. 2002, Gauthier et al. 2007). The *in ovo* levels of both exposure groups are also maximal levels found in American kestrel eggs in southern Ontario (P. Martin, Environment Canada, upubl. data) and peregrine falcon eggs in central and eastern Canada (K.J. Fernie, Environment Canda, upubl. data), and are similar to the concentrations reported in peregrine falcon eggs in the northeastern U.S. (Chen et al. 2008).

The chemical preparation for exposure can be found elsewhere (Fernie et al. 2008). Briefly, the two exposure mixtures were prepared by adding 0.1645 g DE-71 (high exposure) or 0.0351 g DE-71 (low exposure) to 250 mL of the same safflower oil. This resulted in final mixture concentrations of 0.658 μg/μL (high exposure) and 0.140 μg/μL (low exposure). Using three repeatable injecting syringes, i.e., one for each exposure group, the appropriate amount of DE-71 (high: 0.65 μg/μL, low: 0.12 μg/μL) or 100 μL safflower oil for the controls were injected intracranially into day-old frozen-thawed cockerels, which were provided on an *ad libitum* daily diet.

The exposure of the parent kestrels to the commercial pentaBDE mixture, DE-71, or control vehicle, began approximately three weeks prior to pairing (March 17, 2006) and lasted throughout the courtship and incubation phases up until the point where the nestlings (would have) hatched (approximately 75 d). The pairs were housed in breeding pens (1.0 m x 2.4 m x 2.4 m: W x L x H) consisting of two rope perches, a nest box (0.3 m x 0.3 m x 0.4 m: W x L x H), a one-way glass observation window (0.1 m x 0.3 m x 0.01 m: W x L x H), and a food platform (0.15 m x 0.15 m x 0.01 m: W x L x H). The nestlings in this study were only exposed as embryos, i.e., *in ovo* exposure. The care of the birds and all experimental protocols were conducted according to the guidelines of the Canadian Council on Animal Care (Olfert et al. 1993) and approved by the McGill University Animal Care Committee (Appendix A).

Sampling procedures

At 20 days of age, the nestlings were weighed and whole blood samples (0.5 mL) were collected (high $n = 12$, low $n = 15$, control $n = 19$) using a 27 gauge, ½" needle,

between 0900 and 1200 h to control for circadian rhythms in circulating hormones. The plasma from all blood samples was separated by centrifugation and stored at -80°C until completion of the thyroid hormone analysis, specifically total triiodothyronine (TT_3) and total thyroxine (TT_4) concentrations.

Thyroid-stimulating hormone (TSH) challenge

In 2006, when the young were either 17 or 19 d of age, a thyroid-stimulating hormone (TSH) challenge was performed by injecting bovine TSH (Sigma-Aldrich) and then measuring their total triiodothyronine (TT_3) and total thyroxine (TT_4) response levels. This method is a modification for that of testing hypothyroidism in cockatiels (*Nymphicus hollandicus*; Harms et al. 1994). The vials of TSH contained 10 IU, so 10 mL of sterile PBS (phosphate buffered saline) were added to reconstitute the TSH to 1 IU/mL. Aliquots of 1 mL were placed in 1 cc syringes and frozen until use. Nestlings were first weighed (high $n = 12$, low $n = 13$, control $n = 15$) and then an initial blood sample was drawn (0.5 mL) to determine basal TT_3 and TT_4 concentrations. This was followed immediately by an intramuscular injection of bovine TSH, with the volume based on 100µl/100g body weight. Approximately 6 h later (± 5 min), a second blood sample was taken (0.5 mL) for determination of post-TT_3 and post-TT_4 concentrations. To measure the thyroid hormone response of the birds to the TSH injection, the basal TT_3 and TT_4 concentrations were subtracted from the 6 h post-injection TT_3 and TT_4 levels, respectively.

Plasma thyroid hormones

Total triiodothyronine (TT_3) and total thyroxine (TT_4) were measured by radioimmunoassay using the Coat-A-Count human TT_3 kit and canine TT_4 kit purchased from Diagnostic Products Corporation. The sides of the tubes obtained from these ^{125}I-T_3/T_4 solid phase radioimmunoassay kits were coated in monoclonal T_3 or T_4 antibodies and after incubation, the bound and free fractions were separated and the radioactivity was counted using a Canberra-Packard gamma counter E-5002. The radioactivity was counted during 1 min intervals, and the concentrations calculated using a T_3/T_4 standard curve ranging from 2.5 to 60 ng/mL for TT_4 and 0.2 to 0.6 ng/mL for TT_3. Following

validation, the volume of plasma required for T_3/T_4 analysis was reduced by 50% (TT_4: 12.5 μL; TT_3: 50 μL) due to the small amount of plasma available. This was a function of the nonlethal, repeated blood sampling of these small birds, as well as the requirement that the birds be used for additional data gathering, e.g., growth. The analytical methods used for measuring TT_3 and TT_4 concentrations are the same as those used by Fernie et al. (2005).

Morphometric measures

Nest boxes were checked daily, as per Fernie et al. (2009), to determine hatching dates. The morphometric size of each nestling was determined on the day of hatching and subsequently every 5 days (according to the oldest hatchling) until 30 days of age. In American kestrels, most nestlings hatch within 24 h of the first hatchling emerging, and occasionally within 48 h. The morphometric parameters collected included the body mass (recorded to a tenth of a gram using a Ohaus Model ScoutPro SP601 scale), the length of the tibiotarsal (tarsus) bone, the ninth primary right wing feather and the right central rectrix tail feather to the nearest hundredth of a millimeter using digital calipers (tibiotarsus) or to the nearest millimeter using a non-flexible metal ruler (feathers).

Chemical analysis

Daily nest checking also allowed for collection of the first egg of each clutch in order to determine PBDE concentrations. The chemical analysis was conducted according to the same procedure used by Gauthier et al. (2007). The PBDE and hexabromocyclododecan (HBCD) concentrations found in the eggs of the current study, combined with those eggs from 2005, are presented elsewhere (Fernie et al. 2008, 2009).

Statistical analysis

The statistical analyses were performed using the SAS 9.2 analysis program (SAS Institute Inc. 2002-2008). The data were tested for normality and log-transformed when necessary. Differences relating to the sex and age of the birds were identified prior to further statistical analysis. To determine differences in the thyroid hormone (TT_3 and TT_4) levels and the functioning of the thyroid gland (TT_3:TT_4) among exposure groups,

nested one-way analyses of variances (ANOVAs) were used. The nested variable used was the brood of nestlings in order to control for possible differences in sibling or parental care factors. *Post-hoc* pairwise comparisons of least-squared means (LSM) were also performed. Pearson's correlations were completed for the thyroid hormones and the functioning of the thyroid gland with the morphometric size measurements (body mass, length of tarsus and feathers), as well as the *in ovo* BDE concentrations. Statistical significance was determined at $p < 0.05$, and means ± standard errors (SEMs) are presented.

Results

The mean ΣPBDE concentrations found in the first eggs of each clutch collected in 2006 only were 3.14 ± 0.86 ng/g ww for controls, 286.78 ± 48.79 for the low-exposure and 1145.48 ± 123.41 ng/g ww for the high-exposure groups. The congener profile detected in the eggs for 2006 was as follows, reported in decreasing order of their contribution to the ∑PBDE *in ovo* burden concentration: BDE-99 (31%), -153 (18%), -138 (10.5%), -154 (10.5%), -100 (9%), -47 (7%), -209 (6%), -190 (5%), HBCD (1%) and the remaining 2% was made up of BDE-85, -66, -49, -17, -28, 183 in decreasing order. The appearance of HBCD in the egg samples was unexpected and determined to be an exposure artifact during the dosing of the birds (Fernie et al. 2008, 2009).

Total triiodothyronine (TT_3)

There were no significant differences in plasma TT_3 levels between the male and female kestrel nestlings, so the data were combined within treatment regardless of the sex of the nestlings. In nestlings, plasma TT_3 concentrations varied significantly between the ages of 17, 19 and 20 days old. There was a statistically significant difference among the exposure groups for plasma TT_3 levels at 20 d of age when controlling for sibling effects ($F_{18,25} = 2.13$, $p = 0.041$, Fig. 1 and Table 1) but not at 17 or 19 d of age (Table 1). In the nestlings, TT_3 concentrations were significantly and positively correlated with body mass at the ages of 15 and 20 days (15 d: $n = 40$, $r = 0.382$, $p = 0.015$; 20 d: $n = 86$, $r = 0.407$, $p = 0.0001$; Table 2). There were also significant negative correlations between TT_3

levels and *in ovo* ∑PBDE concentrations, BDE-17, BDE-28, BDE-47, BDE-49, BDE-85, and BDE-100 concentrations ($n = 86$, $r \geq -0.212$, $p \leq 0.050$; Table 3).

Total thyroxine (TT_4)

There were no significant differences in plasma TT_4 levels between the male and female kestrel nestlings, and hence the data were combined again. The age of the nestlings also significantly influenced plasma TT_4 concentrations. Significant differences occurred among exposure groups when chicks were 20 days old ($F_{17,22} = 2.37$, $p = 0.030$; Fig. 1 and Table 1) and marginally at 19 days of age ($F_{14,5} = 3.55$, $p = 0.085$; Table 1) when accounting for sibling variation. Compared to control nestlings, low-exposure chicks had significantly lower plasma TT_4 levels at 19 days of age (LSM $p = 0.011$, Table 1) and lower but non-significant levels at 20 days of age. Although there were no significant correlations between plasma TT_4 levels and *in ovo* PBDE or HBCD concentrations, plasma TT_4 levels were positively and marginally associated with the lengths of the ninth primary wing feathers and the central rectrix tail feathers at 15 days of age ($n = 40$, $r \geq 0.290$, $p \leq 0.070$; Table 2).

Functioning of the thyroid gland

The functioning of the thyroid gland was assessed in two manners: the ratio of TT_3 to TT_4 (McNabb 2000) and stimulating conversion of TT_4 to TT_3 through TSH injections. The ratio of TT_3 to TT_4 after TSH injection was similar among treatment groups (Table 1). At 20 days of age and without a TSH injection, there was, however, an influence of the sex of the nestlings on the ratio of TT_3:TT_4. In female nestlings, this ratio was significantly influenced by *in ovo* exposure to DE-71 and when accounting for sibling variation ($F_{16,22} = 6.43$, $p < 0.0001$; Fig. 1 and Table 1). The control female nestlings had significantly lower ratios than the low-exposure females (LSM $p = 0.026$, Table 1) and higher ratios than the high-exposure female chicks (LSM $p = 0.002$, Table 1). For female nestlings overall, the thyroid hormone ratio was positively associated with their body mass at 15 and 20 days of age ($n = 21, 41$, $r \geq 0.411$, $p \leq 0.054$; Table 2) and negatively associated with *in ovo* concentrations of ∑PBDEs, BDE-47, -49, -85, -99, -100, -138, -153, -154 and HBCD ($41 \leq n \geq 24$, $r \geq -0.379$, $p \leq 0.047$; Table 3).

In the male nestlings, the TT_3 to TT_4 thyroid hormone ratio was also significantly different among treatments but only when sibling variation was addressed ($F_{15,23} = 3.61$, $p = 0.003$; Fig. 1 and Table 1). This ratio was not associated with the size measurements of the male nestlings at any age, but was negatively associated with the male nestlings' exposure to *in ovo* concentrations of BDE-47 and -85 ($30 \leq n \geq 29$, $r \geq -0.452$, $p \leq 0.020$; Table 3).

The TSH challenge: post-TSH injection results

After injecting TSH to stimulate conversion of TT_4 to TT_3, there were no significant differences in the post-TT_3 and post-TT_4 levels overall, although there was a significant suppression of the post-TT_4 levels in the high-exposure nestlings compared to the controls (LSM $p = 0.054$, Table 1). The TT_3 and TT_4 responses of the nestlings to the TSH challenge were similar among the treatment groups, although the high-exposure nestlings had a significantly smaller TT_4 response than the control nestlings (LSM $p = 0.037$, Table 1) and the low-exposure nestlings had a marginally significant smaller TT_3 response than the controls (LSM $p = 0.087$, Table 1). There were significant negative associations between the post-TT_4 levels and *in ovo* concentrations of BDE-17, -49, and -190 ($n = 40$, $r \geq -0.337$, $p \leq 0.034$; Table 3), as well as between the TT_4 response and BDE-49, -153, -154, and -190 ($n = 39$, $r \geq -0.309$, $p \leq 0.055$; Table 3). In terms of morphology, nestlings with longer feathers and a greater body mass had higher post-TT_4 concentrations and a greater TT_4 response than nestlings who weighed less and had shorter feathers ($n = 40, 39$, $r \geq 0.334$, $p \leq 0.035$; Table 2).

Discussion

The thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) are important for a number of functions, including reproduction, thermoregulation and growth, with T_3 being 3-10 times more physiologically potent than T_4 (McNabb 2000). In avian species, there is a certain range in which thyroid hormones are optimal for growth, i.e., above or below this range decreases growth (McNabb 2007). The *in ovo* exposure to the pentaBDE mixture, DE-71, was found to influence or was associated with changes in plasma

concentrations of TT_3, TT_4, and the TT_3:TT_4 ratio. The TT_4 levels of the nestlings were suppressed, while the TT_3:TT_4 ratio increased in the female nestlings from the low *in ovo* exposed group. The high-exposure *in ovo* nestlings had a reduced TT_3:TT_4 ratio (females only) and a reduced TT_4 response after the TSH injection. In general, these thyroid hormone parameters were negatively associated with *in ovo* concentrations of various BDE congeners and positively associated with the nestlings' morphometric measurements. The TT_3 levels were positively associated with nestling body mass, and TT_4 concentrations and responses were positively associated with body mass and feather growth of the nestlings.

The thyroid system of vertebrates is important for a number of critical functions including reproduction, thermoregulation and developmental growth (McNabb 2000). The thyroid system is also sensitive to PBDE exposure, and the thyroid system of the nestling kestrels proved sensitive to their *in ovo* exposure to environmentally relevant PBDE levels. The seemingly greater sensitivity of the thyroid system in nestlings from the lower *in ovo* PBDE exposure group compared to the nestlings from the higher exposure was surprising. The reduced TT_4 levels of the low-exposure nestlings may be a function of increased conversion of TT_4 to TT_3, as is reflected in their higher TT_3:TT_4 ratio compared to the controls, especially for the female nestlings. This is also reflected in the higher TT_3 levels, although not significantly so, compared to the controls at 20 days of age. These elevated TT_3 levels in the low-exposure nestlings occur immediately prior to the maximal nestling size (21 d), and TT_3 levels were positively associated with the body mass of the nestlings at the ages of 15 and 20 days. Within the low-exposure nestlings, there appears to be greater conversion of TT_4 to TT_3 resulting in increased TT_3 levels, to levels exceeding the controls, but at the expense of their TT_4 levels. For the majority of growth parameters, low-exposure nestlings had reduced growth (K.J. Fernie, Environment Canada, upubl. data). The elevation in TT_3 levels immediately prior to the timing of the nestlings achieving maximal size may be an attempt at compensation to maintain appropriate thyroid levels for appropriate growth, perhaps as a result of interference by the PBDE contamination. The smaller size of the low-exposure nestlings

is likely a function of the alterations in their thyroid system as reflected by their reduced TT_4 concentrations.

Based on the TT_3:TT_4 ratio results, there appears to be a sex-specific sensitivity in the thyroid system of the nestling kestrels following the *in ovo* PBDE exposure. The results suggest that the thyroid system of female nestlings may be more sensitive to this PBDE exposure, with greater conversion of TT_4 to TT_3 for the low-exposure female nestlings as reflected by their higher TT_4:TT_3 ratios compared to the ratios of the control females, but lower conversion of TT_4 to TT_3 within the high-exposure female nestlings as reflected by their reduced TT_4:TT_3 ratios. The low-exposure nestlings therefore have a more active thyroid system, while the high-exposure nestlings have a less active one. This again supports the hypothesis that there is a disturbance of the proper function of the thyroid gland because of PBDE exposure at environmentally relevant concentrations. It is interesting to note, however, that following the TSH injection, the conversion of TT_4 to TT_3 is comparable for all groups. The exposure groups are therefore able to respond to stimulation in the same manner as the controls.

The administration of TSH increased TT_3 in the exposure groups, reaching levels comparable to those of the controls post-injection. The TT_4 levels following the TSH challenge were, however, affected by PBDE exposure, with the high-exposure nestlings having reduced levels compared to the controls. The thyroid primarily contains T_4 with much less to almost non-detectable levels of T_3 (McNabb 2000). The thyroid gland itself will therefore primarily release T_4. The lower TT_4 levels in the high-exposure group represents a disruption by PBDE exposure as there is less TT_4 for TT_3 conversion, but which results in the maintenance of appropriate TT_3 concentrations for nestling kestrels. The ability to maintain appropriate TT_3 levels is essential as it is physiologically 3-10 times more potent than T_4 (McNabb 2000). Over time, however, the disturbance of the conversion of TT_4 will affect TT_3 levels, as seen in the non-significantly lower TT_3 levels for the high-exposure nestlings at 20 days of age, which, depending on the extent of the disruption, may be detrimental to these nestlings. The extent of the TT_3 and TT_4 response following TSH injection also suggests PBDE interruption of the thyroid system where

both exposure groups had less of a response than the controls, particularly for the TT_4 levels in the high-exposure nestlings and marginally so for the TT_3 levels in the low-exposure nestlings.

Lower thyroid hormone levels have been seen in nestlings exposed *in ovo* to contaminants for a number of species. In two colonies of cormorant hatchlings, the thyroid levels (FT_4, TT_4, TT_3) of the more contaminated colony (2-5x PCBs) were reduced by almost 50% (van den Berg et al. 1994). A single injection of PCBs (0, 0.067, 0.67, 6.7 ppm) at day 0 of incubation caused a reduction in plasma T_4 and T_3 levels at only the highest PCB concentrations of chicken embryos 21 days post-injection (Gould et al. 1999). The *in ovo* exposure to PCBs (mean egg PCB residue: 34.1 μg/g ww) of nestling American kestrels also caused a reduction in plasma TT_3 levels (Smits et al. 2002). The American kestrel PCB study (Smits et al. 2002) could be comparable to what is occurring in this study where there is a reduction in converting TT_4 to TT_3 levels in the high-exposure nestling females. PBDEs have also been found to reduce thyroid hormone levels, as seen in rodents (fetuses, offspring, and dams; Zhou et al. 2001, 2002). In the study by Zhou et al. (2002), DE-71 (0, 1, 10, 30 mg/kg/day) was orally administered to pregnant rats from gestation day 6 to post-natal day (PND) 21. The juvenile rats in the two highest exposure groups had lower plasma TT_4 levels on PND 4 and 14, but no effect on TT_3 levels was seen. This is comparable to the low-exposure female nestlings in this study, where more TT_4 was being converted to TT_3, thereby reducing the TT_4 levels. One study involving the *in ovo* injection (1500 ng/g ∑PBDEs) and developmental (mean 15.6 ± 0.3 ng/g/bird/day) PBDE exposure of nestling American kestrels also caused reductions in plasma TT_4 levels at 36 days of age (Fernie et al. 2005). This is again similar to the current decrease of TT_4 levels in the low-exposure female nestlings, even though the comparable results are from an exposure concentration higher than that of the high-exposure group in this study. The suppression of the plasma TT_4 concentrations in both this study and that of Fernie et al. (2005) indicates the sensitivity of the avian thyroid system to PBDEs, with potentially excessive conversion of thyroxine in order to maintain the more physiologically important triiodothyronine. The physiological stress on the thyroid system, which is critical for growth in birds (McNabb 2000), in conjunction with

PBDE exposure, was likely responsible for eliciting changes in the nestlings' thyroid system.

Further support that exposure to environmentally relevant concentrations of PBDEs affects nestling thyroid levels in birds is found in the various significant negative correlations between PBDE congeners and the affected thyroid hormone levels. Specifically, the altered TT_3 levels at 20 days of age for the exposure groups were associated with many BDE congeners that were measured in the kestrel eggs. Surprisingly, there was not a statistical association with the TT_4 levels and any of the measured BDE congener concentrations. This finding may be a result of those young with the highest BDE concentrations maintaining their TT_4 levels at comparable levels to the controls, since the suppressed TT_4 levels only occurred in the low-exposure groups compared to the control birds. The reduction in the function of the thyroid gland of the high-exposure nestlings, particularly the females, was also associated with various BDE congeners, as were the reductions in the post-TT_4 levels and the response of TT_4 to the TSH injection in the high-exposure nestlings. These negative BDE correlations suggest that reduced thyroid hormone levels and function are associated with higher concentrations of various flame retardant congeners.

PBDEs are therefore of concern in terms of altering the function of the thyroid system, especially in female kestrel nestlings, and hence the thyroid hormone levels, especially TT_3 as no significant correlations were found for TT_4. The reoccurring negative associations with BDE-47, -99 and -100 are of particular concern as the BDE congener concentrations found in wild avian populations generally follow a similar pattern with those particular ones having the highest concentrations; this is true of herring gulls in the Great Lakes Basin (Norstrom et al. 2002, Gauthier et al. 2007) and peregrine falcons in Sweden (Lindberg et al. 2004) and the U.S. (Chen et al. 2008). These BDE congeners were also found to be some of the highest *in ovo* concentrations in the eggs in this study. The association of BDE levels with TT_3 but not TT_4 levels in this study, contrasts with those found in Fernie et al. (2005) in which the TT_4 levels of juvenile American kestrels was associated with the same BDE congeners (BDE-47, -99, -100).

The difference in their exposure routes (embryonic only in this study vs. embryonic and developmental), as well as the longer exposure time to higher PBDE concentrations, may explain the difference in the response of the thyroid hormone systems of the kestrels between the two studies.

Although the congener concentrations measured in the eggs are being used for correlation analysis with nestling hormones in this study, which is a commonly used technique in toxicology studies, the *in ovo* congener concentrations are representative of the maternal levels. Dauwe et al. (2006) found, however, that the majority of the persistent pollutants found in great tit (*Parus major*) nestlings were of maternal origin. An egg injection and developmental exposure to BDE-47, -99, -100, and -153 in juvenile kestrels found that retention times ranged from 7.8 to 45.3% with half-lives ranging from 5.6 to 44.7 d (Drouillard et al. 2007). The congener profile in the great tit nestlings was also found to change over time (from egg to nestling) as a function of metabolism by the nestlings; the BDE congeners found in the highest concentrations, e.g., BDE-47, -99, remained so but lower brominated congeners, e.g., BDE-28, became more important for the older nestlings, i.e., the proportion of the most persistent pollutants decreased over time (Dauwe et al. 2006). This metabolism of the PBDE congeners is likely also occurring in the nestlings of the current study. Here, the lower brominated congeners, e.g., BDE-17 and -28, negatively associated with changes in the thyroid system are becoming more important as disrupting congeners for the nestling kestrel thyroid system. Transthyretin (TTR) is one way in which circulating thyroid hormones are transported (McNabb 2000). It has been found that higher brominated PBDE congeners could not compete with T_4 for transthyretin (TTR) binding (Meerts et al. 2000), which could also explain these lower brominated congeners being negatively associated with alterations to the thyroid system.

It is surprising to note that the altered TT_3 levels and TT_4:TT_3 ratio, as well as the reduced TT_4 response following TSH injection, was statistically associated with *in ovo* HBCD concentrations in the nestlings. Although not intentionally exposed to HBCD, it was an exposure artifact in this study and the levels were measured in the eggs in 2006

only. HBCD has been measured in high concentrations in top predators, i.e., up to 300 ng/g lipid weight in the eggs of guillemot (*Uria algae*): Sellstrom et al. 2003, and up to 2400 ng/g lipid weight in the eggs of peregrine falcons: Lindberg et al. 2004, and are slowly increasing in the environment (Covaci et al. 2006). This current finding is therefore interesting in this regard but requires a more in-depth examination.

There are a number of ways in which the thyroid system could be affected by PBDE exposure. As briefly mentioned above, hydroxylated PBDE (OH-PBDE) metabolites have been shown to compete with T_4 for TTR binding (Meerts et al. 2000; Ucan-Marin et al. 2009) and could be responsible for displacing T_4 and hence causing the lower T_4 levels in the low-exposure nestlings. Thyroid hormone levels could also have been decreased due to an increased clearance from the body by increasing those enzymes responsible for T_4 breakdown and excretion (Zhou et al. 2001). PBDEs, especially their hydroxylated metabolites, have also been found to compete with T_3 for TTR binding (Ucan-Marin et al. 2009), which could explain the altered T_3 levels in this study. Therefore, there exist several possible ways for PBDEs to disrupt the thyroid system and they may not be mutually exclusive. This is, however, beyond the scope of the current study but requires further investigation.

As previously stated, the thyroid hormones are particularly important for proper growth and development of nestlings (McNabb 2000). In avian species, the two hormone groups needed for normal growth after hatching are growth hormones (GH) and thyroid hormones (T_4 and T_3) (McNabb 2000). Exposure of pre-fledging herring gulls to PCBs demonstrated a positive correlation between thyroxine levels and mass, as in this study following TSH injection, although thyroxine levels were not affected by the PCB exposure (Grasman et al. 1996). The *in ovo* and developmental exposure of American kestrels to PBDEs was found to alter nestling growth, whereby the nestlings were larger and gained weight more quickly than the controls (Fernie et al. 2006). This study also reported reductions in TT_4 levels for the PBDE-exposed juveniles (Fernie et al. 2005). In the current study, the altered thyroid system of the nestling kestrels as a function of their exposure to PBDEs may explain the alterations in their growth patterns, which have been

found to be affected by PBDE exposure (K.J. Fernie, Environment Canada, upubl. data). The female nestlings in the low *in ovo* exposure group were lighter in body mass for much of their 30 d growth period and the start of their maximal growth period was delayed (K.J. Fernie, Environment Canada, upubl. data), whereas the high-exposure nestlings were larger and gained weight more quickly, comparable to the findings in Fernie et al. (2006). The positive correlations between body mass and TT_3, and the change in the function of the thyroid gland of the female nestlings, suggests that for low-exposure nestlings, there is excessive conversion of TT_4 to TT_3 in an attempt at compensation to maintain appropriate TT_3 levels to stimulate appropriate growth. Indeed, the body mass of the low-exposure female nestlings are comparable to the controls just after 20 days of age (K. J. Fernie, Environment Canada, upubl. data). There were also positive associations between TT_4 levels and feather lengths reflective of the reductions in TT_4 levels and feather lengths of the low-exposure nestlings (K.J. Fernie, Environment Canada, upubl. data). The high-exposure nestlings were larger (body mass, feathers) and there were positive correlations between the TT_4 response to the TSH injection and body mass and feather growth. These patterns are consistent with the reduced TT_4 response of the high-exposure nestlings compared to the controls, and yet both groups had comparable TT_3 levels critical to growth, which corresponds to the increased body mass and feather lengths of the high-exposure chicks. Those nestlings with altered thyroid hormone levels and function had altered growth as seen with both the low- and high-exposure nestlings, supporting the hypothesis that PBDE exposure altered the thyroid system of the nestling, which in turn has affected their growth.

In conclusion, the thyroid hormone levels of nestling American kestrels, as well as the functioning of their thyroid, were found to be sensitive to PBDE *in ovo* exposure, with the two exposure concentrations affecting the kestrel nestlings differently. The altered thyroid system contributed to the altered growth of the exposed nestlings through inappropriate thyroid hormone levels and thyroid function. The alteration to the thyroid system due to PBDE exposure and hence, nestling growth may also explain the reduced fledging success of these young (Fernie et al. 2009). The thyroid alterations due to *in ovo* PBDE exposure has the potential to be exacerbated in wild avian species with the

continued consumption of PBDE-contaminated food. Changes in the avian thyroid system as a function of exposure to environmentally relevant *in ovo* PBDE concentrations, observed in this study, is cause for concern and further supports the classification of PBDEs as endocrine disruptors.

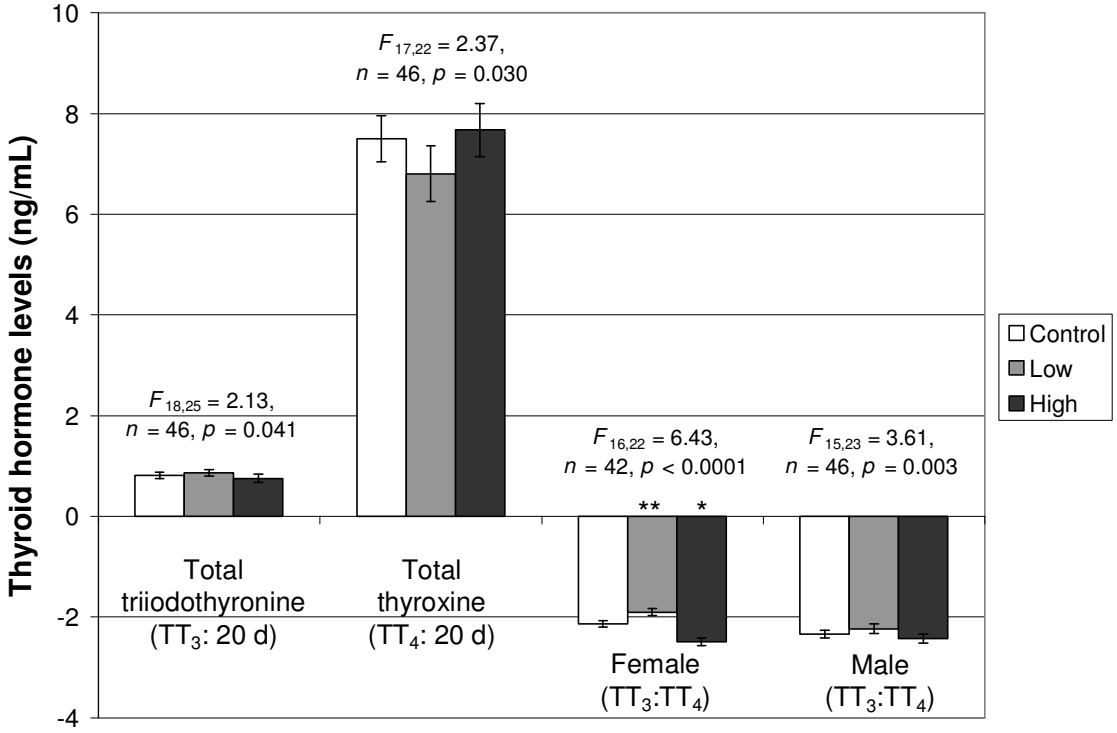

Fig. 1. Thyroid hormone levels differing significantly ($p < 0.05$) among exposure groups for nestling American kestrels (*Falco sparverius*) exposed *in ovo* to PBDEs. LSM significant difference from the controls are indicated where **: $p < 0.01$ and *: $p < 0.05$.

Table 1. Least-squared means of thyroid hormone levels of American kestrel nestlings exposed *in ovo* to PBDEs.

Thyroid hormones (ng/mL)	Age (d)	*n*	Exposure group			Effect of DE-71 exposure (df)[a]	Nested effect of DE-71 exposure (df)[b]
			Control (±SE)	Low (±SE)	High (±SE)		
Total triiodothyronine (TT_3)							
	20	46	0.811 (0.066)	0.869 (0.064)	0.757 (0.077)	N.S.	0.041 (18, 25)
	19	22	0.831 (0.080)	0.892 (0.085)	0.772 (0.083)	N.S.	N.S.
	17	18	1.058 (0.110)	0.849 (0.120)	0.974 (0.126)	N.S.	N.S.
Total thyroxine (TT_4)							
	20	42	7.505 (0.455)	6.807 (0.550)	7.676 (0.529)	N.S.	0.03 (17, 22)
	19	22	10.383 (0.576)	7.086 (0.618)*	9.879 (0.604)	0.024 (2, 5)	0.085 (14, 5)
	17	18	8.754 (0.425)	9.269 (0.462)	10.616 (0.487)	N.S.	N.S.
TT_3:TT_4 ratio							
Female	—	41	–2.137 (0.066)	–1.906 (0.071)**	–2.489 (0.074)*	<0.0001 (2, 22)	<0.001 (16, 22)
Male	—	41	–2.339 (0.080)	–2.233 (0.095)	–2.432 (0.087)	N.S.	0.003 (15, 23)
Post-TSH injection results							
TT_3	19	22	1.138 (0.108)	0.998 (0.116)	0.971 (0.113)	N.S.	N.S.
	17	18	1.241 (0.168)	1.136 (0.183)	1.226 (0.193)	N.S.	N.S.
TT_4	—	40	27.083 (2.081)	22.019 (2.275)	20.788 (2.265)*	N.S.	N.S.
TT_3: TT_4 ratio	—	40	3.099 (0.091)	3.038 (0.099)	2.964 (0.099)	N.S.	N.S.
Post-TT_3 – pre-TT_3	—	40	0.325 (0.042)	0.211 (0.045)(*)	0.285 (0.045)	N.S.	N.S.
Post-TT_4 – pre-TT_4	—	40	17.704 (1.995)	14.081 (2.137)	11.157 (2.127)*	N.S.	N.S.

Note: Unless indicated, there were no differences between the ages or sexes. N.S. = not significant

[a]Statistically significant *p*-values for the one-way ANOVAs. The degrees of freedom are in parentheses.

[b]Statistically significant *p*-values for the nested one-way ANOVAs using the brood of nestlings as the nested factor. The degrees of freedom are in parentheses.

**Signficant difference from the controls ($p < 0.01$).

*Significant difference from the controls ($p < 0.05$).

(*)Marginal significant difference from the controls ($p < 0.10$).

Table 2. Pearson correlation analysis: plasma thyroid hormone concentrations of nestling American kestrels (*Falco sparverius*) exposed *in ovo* to PBDEs and their morphometric measurements at 15 and 20 days of age.

	Thyroid hormone levels									Post-TSH injection thyroid hormone levels					
	Total triiodothyronine (TT_3)			Total thyroxine (TT_4)			Female TT_3:TT_4			TT_4			Post-TT_4 – pre-TT_4		
Morphometric measures	*n*	*r*-values	*p*-values	*n*	*r*-values	*p*-values	*n*	*r*-values	*p*-values	*n*	*r*-values	*p*-values	*n*	*r*-values	*p*-values
Mass (15 d)	40	0.382	0.015	40	N.S.	N.S.	21	0.427	0.054	40	0.334	0.035	39	0.342	0.033
Mass (20 d)	86	0.407	0.0001	82	N.S.	N.S.	41	0.411	0.008	40	N.S.	N.S.	39	N.S.	N.S.
Right ninth primary wing feather length (15 d)	40	N.S.	N.S.	40	0.290	0.070	21	N.S.	N.S.	40	N.S.	N.S.	39	N.S.	N.S.
Right ninth primary wing feather length (20 d)	86	N.S.	N.S.	82	N.S.	N.S.	41	N.S.	N.S.	40	0.419	0.007	39	0.373	0.019
Right central rectrix tail feather length (15 d)	40	N.S.	N.S.	40	0.301	0.059	21	N.S.	N.S.	40	N.S.	N.S.	39	N.S.	N.S.
Right central rectrix tail feather length (20 d)	86	N.S.	N.S.	82	N.S.	N.S.	41	N.S.	N.S.	40	0.447	0.004	39	0.372	0.020

N.S. = not significant

Table 3. Pearson correlation analysis: plasma thyroid hormone concentrations of PBDE *in ovo* exposed nestling American kestrels (*Falco sparverius*) and individual BDE congeners, as well as the ∑PBDE congeners.

Individual BDE congeners	Thyroid hormone levels									Post-TSH injection thyroid hormone levels					
	Total triiodothyronine (TT_3)			Female TT_3:TT_4			Male TT_3:TT_4			TT_4			Post-TT_4 – pre-TT_4		
	n	*r*-values	*p*-values	*n*	*r*-values	*p*-values	*n*	*r*-values	*p*-values	*n*	*r*-values	*p*-values	*n*	*r*-values	*p*-values
BDE-17	86	–0.224	0.023	19	N.S.	N.S.	21	N.S.	N.S.	40	–0.337	0.034	39	–0.284	0.080
BDE-28	86	–0.247	0.022	15	N.S.	N.S.	15	N.S.	N.S.	40	N.S.	N.S.	39	N.S.	N.S.
BDE-47	86	–0.241	0.026	29	–0.418	0.024	27	–0.463	0.015	40	N.S.	N.S.	39	N.S.	N.S.
BDE-49	86	–0.333	0.002	24	–0.465	0.022	21	N.S.	N.S.	40	–0.377	0.016	39	–0.379	0.017
BDE-85	86	–0.226	0.036	27	–0.386	0.047	26	–0.452	0.020	40	N.S.	N.S.	39	N.S.	N.S.
BDE-99	86	–0.195	0.072	32	–0.399	0.024	34	–0.326	0.060	40	N.S.	N.S.	39	–0.284	0.080
BDE-100	86	–0.213	0.049	27	–0.447	0.020	25	–0.383	0.059	40	N.S.	N.S.	39	–0.272	0.094
BDE-138	86	–0.196	0.071	41	–0.379	0.014	41	N.S.	N.S.	40	N.S.	N.S.	39	N.S.	N.S.
BDE-153	86	–0.185	0.088	34	–0.448	0.008	36	N.S.	N.S.	40	–0.274	0.087	39	–0.335	0.037
BDE-154	86	N.S.	N.S.	34	–0.456	0.007	36	N.S.	N.S.	40	–0.281	0.079	39	–0.343	0.033
BDE-190	86	N.S.	N.S.	28	N.S.	N.S.	31	N.S.	N.S.	40	–0.364	0.021	39	–0.309	0.055
HBCD	86	–0.180	0.097	27	–0.435	0.023	25	N.S.	N.S.	40	N.S.	N.S.	39	–0.299	0.064
∑PBDE congeners	86	–0.212	0.050	41	–0.420	0.006	41	N.S.	N.S.	40	N.S.	N.S.	39	–0.287	0.076

N.S. = not significant

FINAL SUMMARY AND CONCLUSION

This study investigated the impact of exposing captive American kestrels to environmentally relevant concentrations of polybrominated diphenyl ethers (PBDEs) on vitamin A (retinol) and thyroid hormones. Retinol and the thyroid system are important for avian reproduction and growth. Both of these parameters were affected by PBDE exposure. Prior to being paired for the breeding season, maternal retinol levels were suppressed in the low-exposed group. These reduced maternal retinol levels were associated with fewer hatchlings and provide a partial explanation for the reduced reproductive success of these birds. Nestling retinol levels were also suppressed by the low *in ovo* PBDE exposure, which were associated with reduced body mass. The nestling kestrels also demonstrated altered thyroid hormone levels that were associated with changes in selected morphometric measurements, i.e., body mass. This offers an endocrinological explanation for the altered growth observed in these nestlings.

PBDEs are an ubiquitous environmental pollutant. In addition to their common occurrence in wildlife, their persistence and lipophilicity are cause for concern, especially for those at the top of the food chain, such as raptors. The importance of PBDEs being found in biota is not fully understood as there are relatively few studies, especially involving retinol, detailing their physiological effects. The results of this study provide an important understanding of some of the physiological changes that help to explain the reduced reproductive success and altered developmental growth in the birds as a function of their exposure to environmentally relevant PBDE concentrations. Through this research, we now have a better understanding of the possible effects of PBDEs on top predatory avian species.

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