Polybrominated diphenyl ethers (PBDEs) are additive flame retardants that are environmentally persistent and bioaccumulative. The developmental effects of in ovo exposure to environmentally relevant levels of the PBDE technical mixture, DE-71, on male reproductive physiology in captive American kestrels (Falco sparverius) was determined. Males were exposed in ovo by direct maternal transfer to DE-71 at three mean concentrations of 289 ng/g ww (low exposure), 1131 ng/g ww (high-exposure), or background levels of 3 ng/g ww (control). As adults, males were paired with unexposed females for breeding and, 1 year later, sacrificed for testes evaluation. While breeding, high-exposure males demonstrated a trend of reduced circulating testosterone levels when their female mate commenced egg laying when compared with controls ($p = 0.056$). No differences in circulating free $T_4$ or $T_3$ were detected. Sperm numbers were elevated on the right testis than controls ($p = 0.034$) with a similar trend for their left testis ($p = 0.055$). High-exposure males had more seminiferous tubules containing lumen than controls ($p = 0.030$), and in proportion to the total number of tubules, low-exposure males had more tubules containing lumen than did controls ($p = 0.016$). Only high-exposure males had fewer than half of tubules containing final spermatids ($43\%$). The results of the present study demonstrate that embryonic exposure to technical DE-71 affects the reproductive tract of adult male kestrels.

**Key Words:** American kestrel; PBDEs; DE-71; testes; testosterone; thyroid.

PBDEs are lipophilic and bioaccumulative brominated flame retardants that have reached global and ubiquitous distribution in the environment, humans, and animals (Hites, 2004). With respect to wildlife, some of the highest levels have been recorded in predatory birds, species that have long been used as sentinels for environmental monitoring. In the common kestrel, *Falco tinnunculus*, for example, PBDE concentrations averaged $12 \ 300 \pm 5 \ 540$ ng/g lipid weight in muscle (Chen and Hale, 2010; Chen et al., 2007), and the eggs of the Great Lakes herring gull, *Larus argentatus*, had average concentrations ranging from 321 to 1191 ng/g wet weight in 2006 (Gauthier et al., 2008). Currently, the levels of penta-BDE congeners are decreasing or reaching a plateau in the eggs of some avian species (Elliott et al., 2005; Gauthier et al., 2007), however, they are still increasing in the eggs of other species such as the peregrine falcon, *F. peregrinus* (Johansson et al., 2009). Production of the penta PBDE mixture (DE-71) ceased in 2006 and currently only the fully brominated decaBDE mixture is used, though is slated for phase out in the United States and Canada and is under review in Europe (www.BSEF.com). However, because the main entry of PBDEs into the environment is thought to occur through their leaching out of end products (Hites 2004), and these products will continue to be a source of environmental and household contamination for an undetermined span of time, the investigation of their physiological effects is of continued importance for wildlife and humans.

PBDEs and some of their metabolites are classified as endocrine disruptors. The DE-71 mixture has a combination of estrogenic and antiandrogenic properties as well as thyroid disrupting capabilities (Hamers et al., 2006), which may be particularly detrimental for the reproductive tract of males. Biomarkers for antiandrogenic effects have been affected in male rats (Kuriyama et al., 2005; Stoker et al., 2004, 2005); notably, a delay in the onset of puberty was observed with developmental exposure to DE-71 (Kodavanti et al., 2010; Stoker et al., 2005, Stoker et al., 2004) and a decrease in androgen-dependent reproductive organ weights (testes and epididymis) with developmental exposure to BDE-99.
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(Kuriyama et al., 2005) but not DE-71 (Kodavanti et al., 2010). Additionally, male rats exposed developmentally to BDE-99 showed an increase in sweet preference, a sexually dimorphic trait normally seen in females, and an accepted biomarker for estrogenizing effects in rats (Lilienthal et al., 2006). Circulating estradiol and testosterone (Lilienthal et al., 2006) were reduced in male rats exposed developmentally to BDE-99, and circulating testosterone appeared to be reduced in rats exposed developmentally to the DE-71 mixture in the highest exposure category (Kodavanti et al., 2010). Thyroid hormones are also critical in regulating seasonal testicular development (Gerlach and Aurich, 2000), including in birds, and PBDEs are thyroid disruptors, causing reduced circulating T₄ in vivo (reviewed in: Costa and Giordano, 2007). However, with developmental exposure to DE-71, male rats recovered from hypothyroidism by 60 days postnatal (Kodavanti et al., 2010).

American kestrels are reproductively sensitive to DE-71 exposure both with diet (Fernie et al., 2008) and developmental exposure (Marteinson et al., 2010), in both cases demonstrating altered courtship behavior frequencies and reduced reproductive success, including fertility. Furthermore, circulating T₄ was reduced in juvenile kestrels that received developmental exposure via egg injection, followed by oral exposure during the nesting phase (Fernie et al., 2005). The objective of the present study was to examine the effects of developmental exposure in ovo to DE-71 at environmentally relevant levels on the male reproductive tract and associated endocrinology in American kestrels. Specifically, sperm numbers and testis mass and histology were assessed as well as circulating thyroid hormone and testosterone levels. The present study is the first to determine the effects of exposure to PBDEs on the reproductive tract in male birds.

MATERIALS AND METHODS

Captive American kestrels (F. sparverius) from the Avian Science and Conservation Centre at McGill University were used in the breeding seasons of 2007 and 2008. Birds were subjected to the natural climate and photoperiod and fed untreated frozen-thawed day-old cockerels (Gallus domesticus) ad libitum. Husbandry of the kestrels was conducted in conformity with the Canadian Council on Animal Care Guidelines (Ollett et al., 1993) and was approved by McGill University’s Animal Care Committee.

The study subjects. The in ovo-exposed males used in the present study were the F₁ progeny of the 2006 dietary exposure F₀ study subjects (Fernie et al., 2008) and are the same individuals for which reproductive behavior and success have been previously reported (Marteinson et al., 2010). Exposure DE-71 levels were environmentally relevant and modeled after concentrations found in the eggs of Great Lakes herring gulls (Gauthier et al., 2008) and European peregrine falcons (Lindberg et al., 2004). Two parental exposure levels of DE-71 dissolved in safflower oil were used, one higher and one lower concentration for which 0.658 µg DE-71/µl safflower oil (0.3 ppm) or 0.140 µg DE-71/µl safflower oil (1.6 ppm) were injected into their food source, respectively. A third group of controls were exposed to the safflower oil vehicle only (for details, see Fernie et al., 2008). Parental (F₀) exposure began at 3 weeks before pairing and continued through incubation until the first chick hatched (average of 75 days). Thus, the present (F₁) study subjects were exposed developmentally during the 28-day embryonic period only, with exposure occurring via direct maternal transfer to the egg. The DE-71 mixture was obtained from the Great Lakes Chemical Company, and the following PBDE congeners were detected in the sibling eggs in decreasing order: BDE-99 (43%), -153 (18%), -100 (15%), -47 (9%), -154 (12%), -85 (1.3%), with the final 2.5% being made up of BDE-66, -138, -49, -190, -209, -28, -17, -183 (Marteinson et al., 2010). Seven males were used from each treatment group (higher, lower, and control) as defined by the exposure categories of their parents (Fernie et al., 2008, 2009) and are categorized as high-exposure and low-exposure herein, as previously described (Marteinson et al., 2010). Due to the low reproductive success of F₀ parents, progeny from 18 pairs were available and so one set of two siblings were used in each group, with the remaining five males being unrelated. Mean 2P3BDE concentrations found in sibling eggs of these individuals were 1131 ± 95 ng/g wet weight for high-exposure and 289 ± 33 ng/g wet weight for low-exposure males and are used as a determinant of approximate embryonic exposure (Marteinson et al., 2010). Background levels of 3 ± 0.5 ng/g wet weight were determined in the eggs of controls. Hexabromocyclododecane (HBCD) was unexpectedly determined in the eggs at low levels of 16 ± 2.6 ng/g wet weight in high-exposure eggs, 3.3 ± 0.7 ng/g wet weight in low-exposure eggs, and 0.002 ± 0.002 ng/g wet weight for controls (Fernie et al., 2008, 2009). The HBCD was not detected in the technical mixture nor in the safflower oil vehicle, thus exposure likely occurred as an artifact during the dosing of the birds, possibly related to background exposure or food levels (Fernie et al., 2009). Detailed descriptions of the chemical analysis and concentrations of PBDEs and HBCD in these eggs are described elsewhere (Fernie et al., 2008, 2009).

In 2007, at 1 year of age, the 21 in ovo-exposed males in the three exposure groups described above were paired with unexposed females and allowed to complete one reproductive cycle (Fig. 1). One year later in the spring of 2008, males in unpaired state were euthanized at 2 years of age during the fertile period for evaluation of the testis at the onset of spermatogenesis.

Measurement of circulating hormone levels. One milliliter of blood was taken at each sampling time point with a heparinized 27.5-gage needle by jugular venipuncture. This frequency and volume of blood withdrawal does not significantly affect reproductive output or hematocrit in kestrels (Rehder et al., 1982). Body mass was recorded prior to withdrawal of each blood sample, and samples were drawn at the same time of day, just prior to feeding between 8:30 and 10:30 a.m. to avoid the effects of diurnal variation in hormone concentrations.

Plasma from the blood samples taken while males were breeding were analyzed for testosterone concentrations at three biological reference points: pairing, the week before the pair laid their first egg and the week that the first egg was laid (Fig. 1). Circulating testosterone (T) concentrations in plasma were determined by enzyme immunoassay (EIA) conducted at Environment Canada’s National Wildlife Research Centre in Ottawa. Salivary testosterone EIA kits (Salimetrics, State College, PA) were used because of their higher sensitivity compared with radioimmunoassay (RIA) kits or serum EIA kits, and thus, less plasma was needed (Washburn et al., 2007), accommodating limited blood sample volumes. The plasma samples were thawed and 15 µl was diluted by 10 times with 135 µl of the assay diluent. The absorbance of this solution was determined using a Molecular Devices plate reader (SpectraMax 190: s/n NN02060). Plates with 96 wells were coated with rabbit anti-testosterone antibodies. Duplicates of each sample were conducted from which a mean was calculated and used for analysis. A calibration curve was prepared with six concentration levels of T standards in duplicates with low and high levels of T within the acceptable range and interassay and intraassay variability was below 10 percent in all cases. The kestrel standards, created using plasma from control birds (not charcoal stripped), were parallel with the salivary standards of the kit. For additional quality control, 12 samples were concurrently analyzed for total T by RIA kits (Siemens, Coat-A-Count, CON6 lot 22), with well-correlated results between the two methods (r² = 0.995), thus validating the results obtained from the salivary EIA.
Plasma was also analyzed for the thyroid hormones T3 and T4 at pairing and five other time points throughout the breeding season to correspond with changes in the natural photoperiod (Fig. 1). The levels of circulating free T3 and T4 in plasma were determined with the use of RIA kits (Siemens Medical Solutions Diagnostics) at Environment Canada’s National Wildlife Research Centre in Ottawa. Plasma samples in duplicates were slowly thawed: 25 l for T4 and 50 l for T3. The T4 levels were analyzed that same day, and the T3 levels were analyzed the next day; all five samples from each individual were analyzed on the same day to reduce variation. The radioactivity was determined with a Canberra-Packard gamma counter E-5002 (serial number 423345) and was counted during 1 min. Results were calculated using linear regression of log–logit representation of the respective calibration curve, prepared with six levels, for both T3 and T4. Commercial controls at various concentrations were also analyzed for quality assurance.

Measurement of sperm numbers during breeding. During egg formation, the spermatozoa from concurrent inseminations become trapped in the perivitelline layer (PVL) surrounding the yolk and can be counted as a reliable indication of ejaculate numbers (Birkhead et al., 1994). The first egg was removed on the day of egg laying and kept frozen until analysis. Once eggs were thawed, the PVL was stretched on a microscope slide, stained with a DNA-binding fluorochrome (Hoechst 33342, Merck), and counts of all sperm nuclei were estimated manually under a Nikon fluorescence microscope (×200 magnification), with the aid of a handheld mechanical counter. The quadrat multiplication technique was used in areas of high density (Birkhead et al., 1994). An additional eight controls from the following breeding year that were found statistically similar to the present controls (t-tests p = 0.66), were also included to increase the sample size (nC = 15), and to reduce the risk of a type 1 error. All low-exposure pairs laid eggs (nL = 7), but only four high-exposure pairs laid eggs (Marteinson et al., 2010), all of which were successfully evaluated.

Evaluation of epididymal sperm numbers, testes mass, and histology. These same individuals were sacrificed 1 year after breeding in early May to coincide with the onset of spermatogenesis (Bird and Lague, 1977) and thus seasonal testicular development in American kestrels. Both testes were extracted within 30 min of death and weighed with an analytical balance, and a ratio of left to right testis was calculated (left testis mass/right testis mass). Body mass was taken just prior to death, and the gonadosomatic index was calculated as the total testes mass × 100/body mass minus testes mass (Bulow et al., 1978).

The left and right epididymis were immediately removed and placed in 3.5 cm3 of phosphate-buffered saline solution at body temperature (40°C). These organs were then thoroughly minced and incubated at body temperature for 30 min to allow sperm to swim out. One drop was placed on an improved Neubauer hemocytometer and, because numbers were low, sperm were counted in all squares. Only swimming sperm could be identified because the samples were contaminated with debris. Four replicates were conducted per individual, from which a mean was calculated.

The larger left testis was selected for histological analysis. Testes were fixed in Bouin’s solution, embedded in paraffin, and 6-μm sections were stained with hematoxylin and eosin. The number of seminiferous tubules containing final spermatids (Fig. 2) was counted on two testis cross-sections from different levels in the testis, under ×670 magnification, from which a mean was calculated for each individual. The numbers of tubules containing lumen

![FIG. 1. Timeline for data collection during each reproductive phase of all three groups of American kestrels. A blood sample was taken for testosterone analysis three times during courtship at the biological reference points of pairing, the week before laying and the week the first egg was laid (△). A blood sample was taken for thyroid hormone analysis at pairing and once per month four times thereafter (○). Behavioral data were collected during the courtship and brood-rearing periods the timing of which is marked by the dashed lines (reported in Marteinson et al., 2010). Body mass was recorded at the same time as all blood samples. The reproductive phases line up in approximately with the timeline of dates but vary between the pairs. In the year following breeding, the same males were euthanized for testis extraction (*).](image1)

![FIG. 2. Cross-section of a seminiferous tubule from the left testis of an unpaired American kestrel at the onset of spermatogenesis. The lumen is visible as are the final spermatids (f.s. →) in the epithelium.](image2)
transformed. Mean hormone concentrations (T, T₃, T₄) and body mass were
for hormone concentrations, the ANOVAs were conducted both with and
using one-way ANOVA with least square difference (LSD) as a post hoc test.
among the groups (repeated measure ANOVA).
also compared for differences in the pattern of change over time using
Pearson’s correlation analyses were conducted
between the physiological parameters measured and the
exposure concentrations of PBDEs as determined in sibling eggs. Statistical analysis was
conducted using SPSS 17.0, all data were tested for normality and homogeneity
of variance, and significance was considered to be

Statistical analysis. For all parameters measured, the high-exposure
(n_H = 7), low-exposure (n_L = 7), and control (n_C = 7) males were compared
using one-way ANOVA with least square difference (LSD) as a post hoc test.
For hormone concentrations, the ANOVAs were conducted both with and
without the covariate of body mass. For proportions, data were arcsine
transformed. Mean hormone concentrations (T, T₃, T₄) and body mass were
also compared for differences in the pattern of change over time using
a repeated measures ANOVA. Pearson’s correlation analyses were conducted
between the physiological parameters measured and the in ovo exposure
concentrations of PBDEs as determined in sibling eggs. Statistical analysis was
conducted using SPSS 17.0, all data were tested for normality and homogeneity
of variance, and significance was considered to be p ≤ 0.05.

RESULTS

Circulating Hormone Concentrations and Body Mass

While breeding, T levels in high-exposure males tended to
be lower than those of controls at the time the first egg was laid
(ANOVA F_{2,18} = 2.10, LSD P_{C,H} = 0.056) (Fig. 3) but did not
differ over time nor at testes extraction. Similarly during
breeding, there was no difference in circulating T₃ or T₄ at any
time, and temporal patterns were similar among the groups.
Testosterone and thyroid levels were not associated with the
in ovo exposure concentrations of PBDEs. There was no
difference in body mass between DE-71 exposed males and
controls at any time.

Sperm Counts

The number of sperm trapped in the PVL of the first egg
produced while males were breeding differed between the three
groups (F_{2,23} = 4.96, p = 0.021). Both the low (LSD P_{C,L} =
0.022) - and high-exposure males (LSD P_{C,L} = 0.028) had higher
PVL sperm counts than controls (Fig. 4). At the time of testes
extraction 1 year later while males were not paired with females,
there was no difference in the concentration of motile sperm in the
epididymis between DE-71-exposed and control males.

Testes Mass and Histology

Two years after their embryonic exposure to DE-71, the
high-exposure males had a heavier right testes than did controls
(F_{2,18} = 2.69, LSD P_{C,H} = 0.034) with a similar trend for the
left testis being heavier (F_{2,18} = 2.16, LSD P_{C,H} = 0.055)
(Fig. 5), and overall, a higher gonadosomatic index than
controls (F_{2,18} = 2.34, LSD P_{C,H} = 0.046). The ratio of left to
right testis mass did not differ between the groups. Testis mass
was positively associated with in ovo exposure concentrations of
ΣPBDEs and the individual congeners, BDE-100, -47, -85,
and -183 (p ≤ 0.046) (Table 1). High-exposure males had more
seminiferous tubules containing lumen than did controls (F_{2,18} =
3.02, LSD P_{C,H} = 0.030) (Fig. 6). Both the low (LSD P_{C,L} =
0.028) had higher

FIG. 3. Plasma testosterone concentrations for breeding male American
kestrels exposed in ovo to higher or lower levels of DE-71 and controls with
error bars representing the SE. Means for the biological reference points of
pairing, the week before egg laying, and the week the first egg was laid are
displayed (p = 0.056). The difference in pattern over time was not different
among the groups (repeated measure ANOVA).

FIG. 4. Counts for all sperm nuclei in the PVL of the first egg in each
clutch of male American kestrels exposed in ovo to DE-71 (n_H = 15, n_L = 7,
n_C = 4). Each column represents the count from one individual; means for
controls (C), low exposure (L) and high exposure (H) are also depicted (∇)
with error bars representing the SE. The eggs of exposed male American
kestrels had more sperm in the PVL than controls (p = 0.021).

Counts for all sperm nuclei in the PVL of the first egg in each
clutch of male American kestrels exposed in ovo to DE-71 (n_H = 15, n_L = 7,
n_C = 4). Each column represents the count from one individual; means for
controls (C), low exposure (L) and high exposure (H) are also depicted (∇)
with error bars representing the SE. The eggs of exposed male American
kestrels had more sperm in the PVL than controls (p = 0.021).
containing final spermatids decreased with increasing in ovo exposure to BDE-47 (Fig. 8), -85, -49, and -28 (p< 0.049) (Table 1).

DISCUSSION

The present study demonstrates that embryonic exposure to high environmentally relevant levels of DE-71 affects male reproductive physiology of American kestrels. Effects were long-term, occurring at 2 years of age, which is the average lifespan of adult kestrels in the wild (Smallwood and Bird, 2002). The results presented here are consistent with those for laboratory rodents exposed developmentally to BDE-99 that demonstrated permanent reductions in circulating testosterone

TABLE 1

<table>
<thead>
<tr>
<th>Congener</th>
<th>% of Left Testis (g)</th>
<th>% of Right Testis (g)</th>
<th>% Tubules with f.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDE-100</td>
<td>14.93</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>BDE-47</td>
<td>8.6</td>
<td>0.49</td>
<td>0.34</td>
</tr>
<tr>
<td>BDE-85</td>
<td>1.32</td>
<td>0.53</td>
<td>0.019</td>
</tr>
<tr>
<td>BDE-49</td>
<td>0.43</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>BDE-28</td>
<td>0.09</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>BDE-183</td>
<td>0.01</td>
<td>0.62</td>
<td>0.044</td>
</tr>
<tr>
<td>ΣPBDE</td>
<td>0.44</td>
<td>0.046</td>
<td>0.021</td>
</tr>
</tbody>
</table>

FIG. 5. Left and right testis mass for control, low, and high in ovo DE-71 exposed male American kestrels where the range, upper, and lower quartile and the median are depicted. Males were in unpaired condition, but testes were collected in May during the fertile period. The left testis of DE-71 high-exposure males was heavier than those of controls on the left (p = 0.055) and right (p = 0.034).

FIG. 6. Number of seminiferous tubules with lumen for control, low, and high in ovo DE-71 exposed American kestrels where the range, upper, and lower quartile and the median are depicted. Males were in unpaired condition, but testes were collected in May during the fertile period. High-exposure males had more tubules with lumen than did controls (P< 0.030).

FIG. 7. The number of seminiferous tubules with standard error bars in cross-sections of the left testis to illustrate the proportion of tubules containing lumen or final spermatids (f.s.) in unpaired male control, low, and high in ovo DE-71 exposure American kestrels. The 50% mark in the total number of tubules in the cross-section is marked with a white line. Both low- and high-exposure males had greater than 50% of the tubules containing lumen. Only high-exposure males less than half of tubules containing f.s. The proportion of tubules containing f.s. was negatively associated with BDE-47, -85, -49, and -28 (p< 0.049).
(Lilienthal et al., 2006) and decreased spermatogenesis (Kuriyama et al., 2005). However, in rats conversely, testis mass was reduced (Kuriyama et al., 2005). The present kestrel study is the first to demonstrate an increase in testicular mass and gonadosomatic index with exposure to PBDEs in vivo in any animal model.

Despite the enlarged testicular mass in high-exposure males, there was no evidence of any associated increase in spermatogenesis. High-exposure males had proportionally fewer tubules with elongated spermatids as their in ovo exposure to specific PBDE congeners increased, and epididymal sperm counts were similar to those of controls. These findings suggest that the increased testicular mass is more likely related to the greater total number of tubules and tubules containing lumen in the exposed males. That the perivitelline sperm counts in exposed males were highly elevated at first appears contradictory. However, these numbers may reflect an increase in ejaculate concentration which can result from reduced copulation frequency (Birkhead, 1991), which was observed for the same individuals in both the low- and high-exposure categories as reported in Marteinson et al. (2010) as well as in the parent kestrels (Fernie et al., 2008). Higher PVL sperm numbers are usually related to an increased number of fertilized eggs (Wishart and Staines, 1999); however, though the high-exposure males had elevated levels of sperm reaching the ovum, they demonstrated reduced egg fertility (Marteinson et al., 2010). This suggests that the sperm of these kestrels may have had decreased fertilization potential, and further research is warranted.

Androgens play a critical role in the reproductive behavior of male birds (Fusani, 2008), such that when their function is suppressed experimentally, reductions in courtship behavior are seen (Grisham et al., 2007). The reduced plasma T levels in the present study are thus consistent with the impaired courtship behavior frequencies recorded in the same individuals (Marteinson et al., 2010), and some of the same PBDE congeners were associated with the reduced androgens found in this study and the reduced courtship behaviors reported in Marteinson et al. (2010). Testis mass was positively associated with increasing in ovo exposure concentrations of ΣPBDE and those of BDE-100, -47, -85, and -183. The proportion of tubules containing final spermatids decreased with increasing in ovo exposure to BDE-47, -85, -49, and -28. In the same males, as reported elsewhere, clutch size, the number of fertile eggs and copulation frequency were also associated with BDE-100, -47, -85, and -183 as well as other congeners including BDE-99, -153, -154, and -138 (Marteinson et al., 2010).

Because males were paired with unexposed females, the alterations in male behavior were implicated in the decreased clutch size and fertility in the same high-exposure individuals (Marteinson et al., 2010). As demonstrated in the present research, the alterations in male reproductive physiology may
have also been a factor in the reduced reproductive success of these same kestrels.

Testis mass is a useful endpoint for assessing the effects of chemical exposure on the functioning and state of the testis (Creasy, 2003). Typically, testes are reduced in size with exposure to chemicals, however, testis mass may also be increased by either enhanced sertoli cell proliferation during testicular development (Defranca et al., 1995) or by increased fluid in the testis (Creasy, 2003). Somatic cell proliferation is regulated in part by triiodothyronine (T3), which inhibits somatic cell differentiation (Defranca et al., 1995). Therefore, hypothyroidism, which is characterized by reduced levels of thyroxine (T4) during postnatal (or posthatch) prepubertal development when somatic cells are proliferating, causes increased final numbers of Sertoli cells, which results in heavier testes that produce more sperm in adulthood (Cooke et al., 1994; Kirby et al., 1996). Several PBDE congeners and their metabolites have the capacity to disrupt the thyroid axis (Hamers et al., 2006). Recently, BDE-47 and more so its hydroxylated analog have been shown to be highly competitive substrates (with T3 and T4) on recombinant gull transthyretin (TTR) and albumin proteins (Ucan-Marin et al., 2010). Hypothyroidism has been recorded in young American kestrels exposed by egg injection and via diet as nestlings to environmentally relevant levels of PBDEs (Fernie et al., 2008). However surprisingly, there was no evidence of any alterations in thyroid function or any subsequent increase in sperm production in the adult kestrels, which usually accompanies hypothyroid enlarged testes (Cooke et al., 1994). Additionally, epididymal sperm counts were not higher in high-exposure males, and the proportion of tubules containing final spermatids was decreased compared with controls. These findings suggest that alterations in thyroid function may be unrelated to the heavier testes of the kestrels exposed to high levels of DE-71. It is possible that a permanent alteration in testicular tissue occurred during embryonic development, however, this does not appear to be sensitive time point for hypothyroid induced increase in adult testis mass in rats (Cooke et al., 1992), which may also be the case in birds.

The second possible mechanism for increased testicular mass is related to a disturbance of the fluid balance in the male reproductive tract that can ultimately result in fluid retention in the testis, increasing its mass (reviewed in: Creasy, 2003). In mammals, this may result from an effect in the efferent tubules, where fluid resorption is inhibited or a blocked, causing a backflow into the testes or because of excessive fluid production by sertoli cells (reviewed in: Eddy et al., 1996). Fluid imbalances are usually characterized by dilated tubule diameter or increased fluid in the intertubular space (Eddy et al., 1996) and are often accompanied by other negative impacts in the seminiferous epithelium or by degeneration of tubules (Eddy et al., 1996). Sex steroids, particularly estrogen, are key regulators of the fluid balance of the mammalian and bird testis, and chemicals that disrupt these pathways can result in increased fluid in the testes (Hess, 2000; Kwon et al., 1997). In the present research, it did not appear as though the tubules were dilated, and there was no degeneration of epithelium. However, the fact that more seminiferous tubules contained a lumen at all in the high-exposure males when compared with controls may be related to the greater testes mass of these males.

**CONCLUSIONS**

The present study demonstrates that embryonic exposure to technical DE-71 at high environmentally relevant levels has long-term (2 year) effects on circulating testosterone, testis mass, and histology of adult male American kestrels. There is no evidence for any alterations in thyroid function in adulthood in these birds nor any evidence for increased spermatogenesis compared with controls, suggesting that a disruption in fluid balance may be a more convincing explanation. However, because testicular histology of the American kestrel has never previously been characterized, and full spermatogenesis was not examined, further research is warranted. The reduced testosterone levels during courtship as well as the reduction in the proportion of tubules containing final spermatids in high-exposure males may be related to the reduced courtship behavior and reproductive success in the same individuals (Marteinson et al., 2010) and are consistent with the findings for laboratory rodents (Kuriyama et al., 2005; Lilienthal et al., 2006). Because exposure concentrations are environmentally relevant and are similar to those recorded in the closely related peregrine falcon (Johansson et al., 2009), and other species (Elliott et al., 2005; Gauthier et al., 2008), wild male birds may be subject to similar alterations of the reproductive tract.

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