Changes in Reproductive Courtship Behaviors of Adult American Kestrels (*Falco sparverius*) Exposed to Environmentally Relevant Levels of the Polybrominated Diphenyl Ether Mixture, DE-71

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Polybrominated diphenyl ethers (PBDEs) are increasing in biota. Here, captive adult American kestrels were exposed daily by diet to safflower oil (controls), or one of two levels of a pentaBDE technical formulation, DE-71 (0.3 or 1.6 ppm), for approximately 75 days, commencing 21 days before breeding. This exposure resulted in eggs having PBDE concentrations similar (low exposure) or within the same order of magnitude (high exposure) reported for wild American kestrels and gulls in the Great Lakes. Compared to controls, kestrels in both exposure groups copulated less, spent less time in their nest boxes, and participated in fewer pair-bonding behaviors. Furthermore, the timing of these behaviors, which is important to creating and maintaining the pair-bond, also differed significantly from the controls. The females in the low-exposure group made fewer compatible trilling calls and ate less frequently. These behavioral changes were compounded by increasing exposure to DE-71 during the 9-day courtship period immediately preceding egg laying, a standard measure of the kestrel courtship period. The birds in the high-exposure group made more food transfers, excited “klee” calls, and copulations, the latter only when compared to the low-exposure birds, whereas the low-exposure males performed fewer pair-bonding behaviors. This study demonstrates that the exposure of kestrels to environmentally relevant levels of DE-71 modifies the quality of the pair-bond, affects the reproductive behavior of both sexes, and occurs when birds are exposed for a short period as adults. In addition, these behavioral effects are consistent with the observed reproductive changes in these birds.

Key Words: DE-71; polybrominated diphenyl ethers (PBDEs); dietary exposure; American kestrels; courtship behavior; birds.

Polybrominated diphenyl ethers (PBDEs) are a class of additive flame retardant materials found in a wide array of commercial, polymeric materials as well as in physical degradation components from these items (e.g., dust, clothing, carpet, and electronic appliances) (Rahman et al., 2001 and references therein). PBDEs are highly persistent and mobile (Rahman et al., 2001) and are ubiquitous in the environment and in biota, especially in consumers at the top of the food chain (de Wit, 2002; Hites, 2004). PBDEs are found in human breast milk, fat, and blood (Damerud et al., 2001 and references therein) and are detected in the eggs of birds of prey such as peregrine falcons (*Falco peregrinus*) (e.g., Lindberg et al., 2004), osprey (*Pandion haliaetus*) (Sellstro¨m et al., 1993), and other predatory birds (Law et al., 2002; Sinkkonen et al., 2004). Some of the highest concentrations of PBDEs, especially BDE-209, were recently reported in common kestrels (*Falco tinnunculus*) in northern China (Chen et al., 2007). Concentrations of congeners arising from pentaBDE formulations (e.g., 2,2’,4,4’-tetrabromoDE [BDE-47] and 2,2’, 4,4’,5-pentabromoDE [BDE-99]) increased between 1981 and 2000 in the eggs of herring gulls (*Larus argentatus*) from sites in the Laurentian Great Lakes of North America (Norstrom et al., 2002). However, a very recent report has shown declines in pentaBDE-derived congeners in herring gull eggs collected in 2004 compared to 2000, at colonial sites on all the five Great Lakes (Gauthier et al., 2007). In addition, several newly detected PBDE congeners, especially hepta- and octabromo diphenyl ethers, were reported in the same herring gull eggs in 2004 (Gauthier et al., 2007). While this research provides an important understanding of the concentrations of PBDEs found in wild birds, the possible impacts of such PBDE concentrations on the reproduction, physiology, and behavior of the birds is not well understood to date.

Increasingly, studies show that PBDEs and their metabolites have the potential to cause adverse effects in laboratory and wild animals. PBDEs are endocrine-disrupting chemicals, particularly impacting the thyroid system (Birnbaum and Staskal, 2004 and references therein). Some PBDE congeners appear to cause neurodevelopmental deficits and have adverse neurobehavioral effects in laboratory studies at concentrations that exceed current environmental concentrations (e.g., Branchi et al., 2002, 2003; Eriksson et al., 2001, 2002; Viberg et al., 2001, 2002, 2003, 2004 and references therein).
2002, 2003). However, the in ovo and developmental exposure of American kestrel nestlings to a combination of pure pentabromoBDE congeners at concentrations currently found in herring gull eggs from the Laurentian Great Lakes (Gauthier et al., 2007; Norstrom et al., 2002) resulted in adverse effects to their thyroid and immune systems, vitamin A levels, glutathione homeostasis, oxidative stress, and growth (Fernie et al., 2005a,b, 2006). Disruption of the thyroid and immune systems, neurodevelopment, and/or neurobehavior may adversely affect an organism’s health. Disruption of the thyroid system may also have serious implications for reproductive success, as various hormonal, physiological, and behavioral events, timed and incorporated properly, are necessary for its occurrence (Wingfield, 1990).

The pentaBDE technical formulation used in this study, DE-71, is one of three types of commercially available PBDE flame retardant products. The Great Lakes Chemical Company (now known as Chemtura) ceased production of the DE-71 mixture in 2004; however, concentrations of the DE-71 congeners are still found in biota. Developmental and behavioral effects in fish occurred following controlled exposure to DE-71. The 7-day embryonic exposure of estuarine killifish minnows (Fundulus heteroclitus) to very low concentrations of DE-71 (0.001 and 100 μg/l) altered their activity level, fright response, predation rates, and learning ability in subsequent life stages (Timme-Laragy et al., 2006). Reproductive and associated behavioral effects from exposure to DE-71 have yet to be determined in any wildlife species.

We have conducted a multigenerational study exposing captive American kestrels (Falco sparverius), a small bird of prey, to environmentally relevant levels of DE-71 in order to determine if such exposures would affect their reproduction, physiology, and behavior. Given that PBDEs can elicit endocrine-disrupting activities (important in development and growth) and that the thyroid system of American kestrels in particular has been affected by PBDE exposure (Fernie et al., 2005a), it follows that their behavior may also be affected, particularly those behaviors modulated by the thyroid system. Further support for this hypothesis derives from the change in courtship and incubation behavior that was associated with altered reproductive success when American kestrels were exposed to environmentally relevant levels of polychlorinated biphenyls (PCBs) (Fisher et al., 2001, 2006). The objective of the present study was to determine and compare potential changes in reproductive behaviors of captive American kestrels associated with their exposure to environmentally relevant concentrations of DE-71, particularly during the critical courtship period.

MATERIALS AND METHODS

This study was conducted at the Avian Science and Conservation Centre of McGill University (Montreal Quebec) using captive American kestrels of known age and pedigree. Male and female adult kestrels were randomly assigned to one of three groups: the high DE-71 exposure group (1.6 ppm; n = 11 pairs in 2005 and n = 10 pairs in 2006), the low DE-71 exposure group (0.3 ppm; n = 10 pairs in 2005 and n = 10 pairs in 2006), or the control group (n = 10 pairs in 2005 and n = 11 pairs in 2006). Each group of kestrels was fed ad libitum on their regular diet of frozen-thawed day-old cockerels.

Exposure and Dosing Concentrations of DE-71. Based on the higher concentrations of PBDE residues found in the eggs of herring gulls collected at colonies in the Great Lakes Basin in 2000 or 2004 (Gauthier et al., 2007; Norstrom et al., 2002), two dosage levels were determined that would result in the kestrels laying eggs with current, environmentally relevant residue levels. The kestrel adults were exposed to DE-71 through their diet, beginning on 26 March 2005 and then the following year on 17 March 2006. This exposure began 3 weeks prior to pairing and continued through the courtship, egg laying, and incubation periods for approximately 75 days in each year or until the first chick (would have) hatched. This temporal pattern of exposure would reflect environmental and physiological conditions experienced by American kestrels and herring gulls in southern Ontario and the Great Lakes.

In this study, the DE-71 mixture was obtained from the Great Lakes Chemical Company and protected against potential photocatalytic degradation throughout. The preparation of the dosing solutions was identical in 2005 and 2006, with minor differences in the concentrations because the DE-71 was highly viscous. Each of the three solutions were prepared using 250 ml of the same safflower oil (Master Choice, 100% pure): the high-exposure treatment involved 0.1645 g of DE-71 technical mixture mixed with safflower oil to produce a final concentration of 0.658 μg/μl; 0.0351 g of DE-71 was added to safflower oil to produce 0.140 μg/μl for the low-exposure level; only safflower oil, prepared identically, was used for the control exposure. The mixtures were then stirred slowly for 20 h in brown bottles lightly covered with aluminum foil until the DE-71 dissolution (when appropriate), and outgassing of the n-hexanes was complete.

Three repeatable injecting syringes, one exclusive to each treatment, were used to inject daily the solutions into the brains of day-old frozen-thawed cockerels. Based on a mean cockerel weight of 40.4 g, and using an injection volume of 100 μl (or 0.1 ml), 12 μg of the DE-71, or 0.12 μg/μl, was injected into each cockerel for the low-exposure birds to receive an estimated dietary exposure of 0.3 ppm, whereas 60 μg, or 0.65 μg/μl, was injected into each cockerel to create an estimated dietary exposure of 1.6 ppm for the high-exposure treatment. These exposure levels may be underestimated for the second year of this study as the PBDE half-lives in adult American kestrel are expected to range from 72 to 572 days (Drouillard et al., 2007). Contaminant levels in the cockerel were reflected by the control eggs (e.g., this study, Fisher et al., 2001). Each pair of adult male and female kestrels received three dosed cockerels per day until their chicks hatched; dosing of individuals by gavage was impossible since it would have prohibited reproduction in this species.

The first egg produced by each pair of kestrels was collected and used for determining chemical concentrations. The chemical analysis of the eggs followed the methodology used in Gauthier et al. (2007). In addition, a sample of the DE-71 mixture used to create the two dosing solutions fed to the kestrels were analyzed for 11 brominated dioxin and brominated furan congeners by Axys Analytical Services (Sidney, British Columbia, Canada). Samples were spiked with 13C/12C-labeled standards added to the samples.

Animal husbandry. The kestrels were paired on 21 April 2005 (n = 31 pairs) and in the following year on 7 April 2006 (n = 31 pairs). Each bird had previous breeding experience and was paired with another bird that was genetically unrelated within the past six generations. In 2006, the birds remained in their same treatment groups but were paired with a different bird than they had been with in 2005. The pairs were housed in breeding pens (1.0 × 2.4 × 2.4 m) equipped with a nest box (0.3 × 0.3 × 0.4 m). The pens also contained two small perches, a one-way glass observation window (0.1 × 0.3 × 0.01 m), and a food platform (0.15 × 0.15 × 0.01 m). The treatment and care of the kestrels were conducted in accordance with the Canadian Council on Animal Care guidelines (Olfert et al., 1993).
**Behavioral observations.** Behavioral observations of the pairs began on 22 April 2005 and ended on 4 June 2005. The following year they began on 8 April 2006 and ended on 23 May 2006. The observations lasted approximately 6 weeks from the time the birds were paired, usually until their clutch of 4–6 eggs had been completed. In 2005, the pairs were observed 6 days a week for a total of 38 days. In 2006, the pairs were observed 7 days a week for a total of 46 days. The pairs were observed twice daily in random order, once in the morning and then again in the afternoon, with each pair being monitored for 2 min at 15-s intervals. The behaviors performed by each kestrel at each 15-s interval were recorded (Table 1).

American kestrels perform various behaviors specifically during the prenesting period which are critical for mate choice and pair-bonding (Balgooyen, 1976; Duncan and Bird, 1989; Fisher et al., 2001, 2006; Palokangas et al., 1992; Villarroel et al., 1998; Willoughby and Cade, 1964). Mate choice, and the establishment and maintenance of the pair-bond between the sexes, is based on nest box inspections, important for sexual stimulation and establishing an acceptable nesting site (Duncan and Bird, 1989; Willoughby and Cade, 1964); copulations that are important for fertilization and potentially inferring mate quality (Villarroel et al., 1998; Willoughby and Cade, 1964); and food transfers that demonstrate the male’s provisioning ability and help the female to achieve sufficient condition for laying and incubation (Duncan and Bird, 1989; Palokangas et al., 1992; Villarroel et al., 1998; Willoughby and Cade, 1964). Kestrels make several vocalizations, including excited “klee” calls and sociable “chitter-whine” calls (Willoughby and Cade, 1964). A male kestrel will posture to gain the attention of the female, by puffing his feathers, repeatedly rotating his head, and pumping his body. He also performs aerial displays to mark his territory, attract mates, and repel competition (Duncan and Bird, 1989; Palokangas et al., 1992; Willoughby and Cade, 1964).

**Statistical analysis.** The kestrels exposed to the DE-71 showed significant delays in clutch initiation (the laying of the first egg) compared to the control birds (Fernie and Shutt, unpublished data). Consequently, two temporal perspectives were used to analyze the courtship behavioral data: the Julian date and nesting chronology (Fisher et al., 2001). The Julian date involved calendar time periods of 5 days per period, from the time of pairing until clutch initiation, and maintained the same length of exposure to DE-71 across all the birds but without regard for the differences in their reproductive stages during this prelaying period. The nesting chronology method focused on the standard courtship period of the 9 days immediately prior to clutch initiation (Smallwood and Bird, 2002) and thereby controlled for the reproductive period but not the length of exposure to the DE-71. Only the pairs that laid a clutch of four or more eggs and were paired at the beginning of the experiment each year were used for the statistical analysis (N = 19 control pairs; N = 17 low-exposure pairs; N = 15 high-exposure pairs).

The observations were organized into functionally similar categories since many of the behaviors occurred infrequently (Table 1) (Fisher et al., 2001). For the Julian date method, means were calculated for behaviors performed by each bird or pair during each 5-day block in the prenesting period and then analyzed using repeated-measures (RM) ANOVAs with least squares means (LSMs) post hoc tests. For each bird or pair, cumulative means were also calculated for their various behaviors in the prenesting period. For the nesting chronology, each type of behavior was summed, and then a mean for the behavior was calculated for each bird/pair during the 9-day courtship period. These prenesting and courtship data were analyzed using nonparametric or parametric one-way ANOVAs with least squares means post hoc tests. With one exception, there were no statistical differences between years; otherwise, the analysis was conducted separately for each year. The level of significance was p < 0.01 since possible biological effects of exposure to DE-71 were too important to overlook by committing a type I error. The increased significance level increased the power of the tests to better identify small biologically significant effects and reduced the chances of stating that there were no effects when there were truly biological effects (Hayes, 1987; Zar, 1996).

**RESULTS**

**Concentrations of PBDE Congeners**

PBDE concentrations in the eggs were similar within each exposure group between 2005 and 2006, so data were combined for both years within each treatment group. The kestrel laid eggs with the following mean concentrations of total PBDE congeners: control eggs, 3.01 ± 0.46 ng/g wet weight (ww) (N = 19; mean ± SEM); low-exposure eggs, 288.60 ± 33.35 ng/g ww (N = 17); high-exposure eggs, 1130.59 ± 95.34 ng/g ww (N = 16). The sum concentrations of the seven major congeners (BDE-28, -47, -100, -99, -154, -153, and -183) were 2.07 ± 0.29 ng/g ww in the same control eggs, 276.24 ± 32.25 ng/g ww in the low-exposure eggs, and 1062.41 ± 92.11 ng/g ww in the high-exposure eggs. There were nondetectable or nonquantifiable levels of the 11 brominated dioxins and brominated furans in the DE-71 mixture fed to the kestrels.

**Behavioral Changes from Pairing to Clutch Initiation (Julian Date)**

In this section, the length of exposure to DE-71, but not the reproductive status, was similar among the kestrel pairs from pairing until egg laying commenced. The copulatory behavior of the kestrels was affected by exposure to DE-71 (RM ANOVA: treatment effects F8,26 = 2.99, p = 0.02) (Fig. 1). When the birds were first paired, the control pairs copulated more frequently than either the low- or high-exposure pairs (ANOVA: F2,16 = 9.59, p = 0.002). The frequency of copulations changed over time (F4,13 = 10.26, p = 0.0006), but this temporal change was altered differentially by the levels of DE-71 exposure (treatment × time interactions F8,26 = 2.99, p = 0.02). In the 5 days immediately preceding egg laying, the control pairs had copulated more often, cumulatively, than the pairs in either of the exposure groups (ANOVA: F2,16 = 4.59, p = 0.03; LSM ps ≤ 0.02).

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**TABLE 1**

<table>
<thead>
<tr>
<th>Functional category</th>
<th>Behaviors</th>
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<tr>
<td>Sexual behaviors involving</td>
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<td>the pair</td>
<td>Proximity (within 15 cm of each other)</td>
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<td>Food transfers</td>
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<td>Male sexual pair-bonding</td>
<td>Aggression</td>
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<td>behaviors</td>
<td>“Whine” and/or chitter-whine calls</td>
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<td>Nest box inspections</td>
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<td>Male posturing</td>
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<td>Female sexual pair-bonding</td>
<td>Whine and/or chitter-whine calls</td>
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<td>Female posturing</td>
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<td>Male/female flight behaviors</td>
<td>Aerial displays</td>
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<td>On the grill of the breeding pen</td>
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<td>Male/female food consumption</td>
<td>Eating</td>
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<td>Male/female body care</td>
<td>Preening</td>
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<tr>
<td>Male/female inactivity</td>
<td>Perching</td>
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<tr>
<td>Male/female vocalizations</td>
<td>Excited klee call</td>
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Subsequently Used for Statistical Analyses
The pair-bonding behaviors and nest box inspections of the kestrels were also influenced by their exposure to DE-71. Nest box inspections by the male kestrels differed over time depending on their level of exposure to DE-71 (treatment $\times$ time interactions $F_{8,24} = 2.01$, $p = 0.08$), and there were potential overall effects from the DE-71 exposure that warrant further investigation (treatment effects $p = 0.14$) (Fig. 2). As egg laying approached, the control males spent significantly more time in their nest boxes and participated in more pair-bonding behaviors than the males from either of the treatment groups (nest boxes: LSM $p < 0.03$, Fig. 2; pair-bonding: LSM $p < 0.10$, Fig. 3). Similarly, during the same time period, the control females also spent more time in their nest boxes than the high-exposure females (LSM $p = 0.07$).

Food consumption, essential for egg laying, was marginally affected by the DE-71 exposure (RM ANOVA treatment effects: males $p = 0.12$; females $p = 0.14$). Eating patterns of the male kestrels varied significantly across time depending on their level of exposure (treatment $\times$ time interactions $F_{8,24} = 2.03$, $p = 0.09$) (Fig. 4A). The exposed males generally ate more often than the control males throughout the prelaying period, significantly so for the low-exposure males initially after pairing (LSM $p < 0.05$) and the high-exposure males during the third time period (LSM $p = 0.08$). In contrast, the exposed females generally ate less than the controls shortly after pairing, and at specific periods, the low-exposure females ate significantly less than the controls (second period: LSM $p = 0.09$) or the high-exposure females (third period: LSM $p = 0.09$) (Fig. 4B).

**Changes in Behaviors during the 9-Day Courtship Period (nesting chronology)**

In this section, the reproductive phase, but not the length of exposure to DE-71, was similar for all the birds. The differences in courtship behaviors from pairing until egg laying continued during the critical 9-day courtship period immediately preceding egg laying. The frequency of food transfers was affected by the high concentrations of DE-71 ($F_{2,48} = 5.77$, $p = 0.01$) (Fig. 5A): High-exposure pairs performed more food transfers than the controls (LSM $p = 0.01$) or low-exposure pairs (LSM $p = 0.002$). Of note, in a pairwise comparison of the copulatory rates, the high-exposure pairs copulated twice as often as the low-exposure pairs ($F_{1,31} = 2.75$, $p = 0.10$) (Fig. 5B). The post hoc tests
indicated that the low-exposure females made fewer compatible trilling calls (LSM \( p = 0.07 \)) than the control females.

In 2005, exposure to DE-71 had significant effects on the frequency of klee calling (\( F_{2,23} = 2.94, p = 0.07 \)) and pair-bonding behaviors (\( F_{2,23} = 3.19, p = 0.06 \)) by the male kestrels during the courtship period. Male kestrels exposed to high levels of DE-71 made more excited klee calls than the control (LSM \( p = 0.05 \)) or low-exposure males (LSM \( p = 0.04 \)). The low-exposure DE-71 males performed fewer pair-bonding behaviors than the control males (LSM \( p = 0.02 \)) (Fig. 5C).

**DISCUSSION**

American kestrels were exposed to one of two levels of DE-71, resulting in the kestrels laying eggs containing environmentally relevant levels of PBDE congeners. In southern Ontario (Canada), wild kestrel eggs had concentrations of sum PBDEs ranging from 15 to 198 ppb ww in 2003 (P. Martin, Canadian Wildlife Service); in Great Lakes herring...
gull eggs, mean concentrations of sum PBDEs ranged from 293 to 665 ng/g ww in 2004 (Gauthier et al., 2007). The PBDE concentrations in the captive kestrel eggs are similar (low-exposure group) or within the same order of magnitude (high-exposure group) as these species.

The reproductive success of birds is modulated by multiple factors, including mate quality and compatibility, the strength of the pair-bond, and food provisioning by the male. During the prelaying period, many of these factors are evaluated through the frequency and timing of courtship behaviors. Environmental contaminants have affected the reproductive behavior and success of birds (e.g., Fernie et al., 2001; Fisher et al., 2001, 2006; Fox et al., 1978). In this study, the exposure of American kestrels to environmentally relevant levels of DE-71 affected the timing and frequency of many courtship behaviors that are important for establishing and maintaining the pair-bond, successfully laying eggs of sufficient size and quality to maximize hatching success and, therefore, ultimately reproductive success. The changes in courtship behaviors of the birds exposed to either level of DE-71 are consistent with the observed changes in their reproductive success (Fernie et al., unpublished data).

One of the first steps in kestrels establishing a strong pair-bond is frequent copulations allowing each bird to assess the quality and compatibility of their potential mate (Villarroel et al., 1998; Willoughby and Cade, 1964). During this critical assessment period, the control birds copulated three times more frequently than the birds in either treatment group. This reduction in copulatory activity began after only 21 days of exposure, including the low-exposure birds that laid eggs having levels currently found in wild kestrels and gulls. The effects of the DE-71 on suppressing copulatory behavior continued as egg laying approached, with the treatment pairs experiencing a more rapid suppression of copulatory rates, especially the low-exposure pairs. Overall, the control pairs copulated more frequently than either treatment group, and surprisingly, the high-exposure pairs copulated more often than the low-exposure pairs. This study indicates that the exposure of kestrels to DE-71, but not to environmentally relevant high levels of PCBs (Fisher et al., 2001), affects the copulatory behavior of birds. It also contrasts with the lack of effects on sexual behavior of male rats developmentally exposed to PBDE-99 (Kuriyama et al., 2005). Furthermore, reductions in the copulatory activity from exposure to DE-71 may reduce the quality of the pair-bond and the fertility of eggs.

Food transfers from the male to the female bird are another mechanism for establishing and maintaining the pair-bond, through evaluating mate quality including the provisioning ability of the male. The female’s acceptance of the male’s food offer reflects her receptiveness to him (Duncan and Bird, 1989; Palokangas et al., 1992; Villarroel et al., 1998; Willoughby and Cade, 1964). Here, the frequency of the courtship food transfers was affected differentially by exposure to the two levels of DE-71. Male kestrels in the high-exposure group made more food transfers than the control or low-exposure males, and the high-exposure females were receptive of these additional food transfers. The increased food transfers of the high-exposure pairs reflect a similar pattern observed in kestrels exposed to PCBs (Fisher et al., 2001) and provides further support of qualitative differences in the pair-bond among treatments that would affect reproductive success.

In American kestrels, mate selection and establishment of the pair-bond also involves the male posturing toward the female, joint nest box inspections, and specific calls reflecting mate compatibility (Smallwood and Bird, 2002). The male postures to solicit copulations, transfer food, and inspect the nest box with the female and his sexual behaviors play a critical role in stimulating female sexual behaviors and the onset of egg laying (Duncan and Bird, 1989; Willoughby and Cade, 1964). In this study, the frequency of pair-bond behaviors and nest box inspections by the treatment males differed from the controls. Initially, the controls made more copulations but fewer nest box inspections and pair-bonding behaviors; as egg laying approached, they completed more nest box inspections, pair-bonding behaviors, and copulations. The opposite behavioral patterns were seen in the birds exposed to DE-71, and their reduction in nest box inspections contrasts with the increased nest box activities of male kestrels exposed to PCBs (Fisher et al., 2001). Furthermore, compared to controls in this study, high-exposure males made more excited klee calls and fewer pair-bond behaviors, as did the low-exposure males whose female mates made fewer compatible calls. Together, these results suggest that exposure to DE-71 adversely affects the establishment and maintenance of the pair-bond.

The changes in the critical timing of the courtship behaviors resulting from the exposure to DE-71 would likely also delay egg laying, reduce the fertility of the eggs, and negatively impact other clutch parameters determining reproductive success. As uptake of the DE-71 continued following pairing, the timing of the courtship behaviors by the treatment birds differed markedly from that of the control birds. Exposure to both levels of DE-71 changed the appropriate timing of the birds’ copulatory behavior, nest box inspections, pair-bonding behaviors, and food consumption patterns. Perhaps, most critically, as the birds were continuously exposed to DE-71 during the courtship period, the treatment birds failed to copulate, participate in pair-bonding sexual behaviors, and investigate their nest boxes, at appropriate times nor as frequently as the control birds. Given the changes in the timing and frequency of these courtship behaviors of the treatment birds, the birds exposed to the DE-71 would likely experience delays in clutch initiation which was observed (Fernie and Shutt, unpublished data).

The changes in the reproductive courtship behaviors of the PBDE-exposed kestrels may reflect changes in the production and/or concentrations of reproductive hormones (e.g., testosterone, estradiols, luteinizing hormone [LH]). While developmental exposure to BDE-99 had no affect on the testosterone
and LH levels of male rats (Kuriyama et al., 2005), sum PBDE levels were associated with circulating progesterone but not basal testosterone levels of incubating male glaucous gulls (Larus hyperboreus) (Verrault et al., 2006). The similarities (increased food transfers) and differences (nest box activity, copulations) in the changes to avian courtship behaviors from exposure to PBDEs (here, 0.3 and 1.6 ppm) versus PCBs (34 ppm in Fisher et al., 2001) may reflect toxicity differences of these chemicals regarding endocrine disruption, Aryl-hydrocarbon-mediated systems, and neurobehavioral toxicity (e.g., Chen and Bunce, 2003). This comparison also demonstrates that low levels of PBDEs relative to PCBs can elicit changes in reproductive behavior of birds.

Food consumption by a female bird during the prenesting period is critical to the timing of egg laying, the quality and size of the eggs produced, and the number of eggs laid. The low-exposure females ate less frequently than the control females following pairing and, shortly thereafter, less frequently than the high-exposure females. Furthermore, the females in both exposure groups did not eat as frequently as the control females immediately prior to clutch initiation. Unfortunately, the birds were not weighed from the time of pairing until mid-incubation in order to minimize disturbance and maximize reproductive success. The reduction in food consumption, especially by the low-exposure females, may have contributed to the delays in clutch initiation and reductions in egg size (Fernie and Shutt, unpublished data).

In previous studies, the exposure of various animals to PBDE congeners altered activity levels and responses. In this study, the overall activity of the kestrels was affected by their dietary exposure to DE-71 as adults for a minimum of 21 days. The high-exposure birds were more active overall, making more food transfers, copulations, and excited klee calls; the low-exposure birds were less active overall, performing fewer pair-bonding behaviors, copulations, and “trill” calls. These patterns are consistent with the initial hypoactivity and subsequent hyperactivity observed in adult mice and rats that were exposed in utero or neonatally to similar or higher concentrations of BDE-153 (postnatal day [PND] 10 oral exposure to 0.45, 0.9, and 9 mg/kg of body weight [bw]; Viberg et al., 2003), BDE-99 (300 μg/kg in utero on gestational day [GD] 6; Kuriyama et al., 2005) (PND 10 oral exposure to 8 mg/kg bw; Viberg et al., 2002) (0.8 or 12 mg/kg bw at PND 10 for male mice, Eriksson et al., 2001), or BDE-47 (10.5 mg/kg bw at PND 10 for male mice, Eriksson et al., 2001). Mice exposed in utero and as pups (GD 6 to PND 21) were hyperactive as juveniles but then became hypoactive as adults (0.6, 6, or 30 mg/kg per day) (Branchi et al., 2002). The embryonic exposure of estuarine minnows (F. heteroclitus) to lower levels of DE-71 (0.001 and 0.01 μg/l from day 0–7 postfertilization) resulted in hypoactivity and elimination of the normal fright response with potential impacts on predator recognition and avoidance (Timme-Laragy et al., 2006). Neurobehavioral alterations occurred when mice were exposed during, but not after, the critical period of neonatal brain development (Eriksson et al., 2002). Our study indicates that the exposure of birds, as adults, to environmentally relevant levels of DE-71 is sufficient to induce behavioral changes early in the reproductive season.

In conclusion, the timing and frequency of courtship behaviors of captive American kestrels were adversely affected by their exposure to DE-71. These captive birds laid eggs with PBDE concentrations similar to those recently found in the eggs of wild kestrels and herring gulls in the Great Lakes. Some courtship behavioral changes associated with the DE-71 exposure were similar to or contrasted with those observed in kestrels exposed to higher concentrations of PCBs. In this study, the pair-bond between male and female birds was compromised through changes in the timing and frequency of behaviors of both sexes (i.e., they were notmediated predominantly through one sex only) exposed to DE-71 as adults. The behavioral modifications seen in both treatment groups are consistent with their delayed clutch initiation and reductions in egg size, fertility, and reproductive success (Fernie and Shutt, unpublished data). These compromises in reproductive behavior are likely occurring in wild birds exposed to similar levels of PBDEs. Future research should focus on possible changes in reproductive and predator behavior, reproductive hormones, as well as energetic expenditures, of birds in multiple generations exposed to PBDEs.

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REFERENCES


