Reproductive and Growth Changes in American kestrels (*Falco sparverius*) Exposed to Environmentally Relevant Levels of DE-71.

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Introduction.

Polybrominated diphenyl ethers (PBDEs) are manufactured as additive flame retardants in the production of polyurethane foams, textiles and plastics. PBDEs are persistent, lipophilic compounds that bioaccumulate through to the highest levels of the food chain (e.g., Lindberg *et al*., 2004). Recent reports have demonstrated that avian populations are subject to PBDE bioaccumulation but that temporal trends differ among species (Norstrom *et al*., 2002, Sellström *et al*., 2003). PBDE concentrations peaked in Guillemot (*Uria algae*) eggs in the Baltic Sea during the mid- to late-1980s (Sellström *et al*., 2003), whereas PBDE concentrations have increased exponentially in wild birds in the North American Great Lakes from 1981 to 2000 (Norstrom *et al*., 2002). Eggs of wild peregrine falcons (*Falco peregrinus*) in Sweden (Lindberg *et al*., 2004) and Norway (Herzke *et al.*, 2005) contain among the highest concentrations (39,000 ng/g lw in Sweden) of ΣPBDEs measured in wildlife; concentrations of BDE-99, -100, -153, -154, and -209 significantly increased in peregrine falcon eggs collected in Greenland between 1986 and 2003 (Vorkamp *et al.* 2005).

Exposure of wild birds to commercially available brominated flame retardant mixtures and PBDEs is of concern since these and other structurally similar chemicals (e.g., polychlorinated biphenyls or PCBs) have been shown to alter reproductive and physiological systems in vertebrates. Bromkal 70–5DE, a technical PeBDE product, has demonstrated weak dioxin-like activity in studies with rat hepatoma cells (Hanberg *et al.*, 1991). Induction of phase I and phase II detoxification liver enzymes have also been demonstrated in rats exposed to technical PBDE products or brominated mixtures (Fowles *et al.*, 1994). Exposure to PBDEs and PCBs has altered blood thyroid hormone homeostasis (Darnerud *et al*., 2001; McDonald, 2002) and/or vitamin A stores (Hallgren *et al*. 2001; Rolland, 2000). Changes in the thyroid system and retinol stores may alter the development, immuno-competence, reproductive success, and other physiological processes in birds. Previous research in our laboratory found that exposure to BDE-47, -99, -100, and -153 at environmentally relevant concentrations affected the growth (Fernie *et al.*, 2006), thyroid hormones and retinol (Fernie *et al.*, 2005a), as well as immune function (Fernie *et al.*, 2005b) of captive juvenile American kestrels (*Falco sparverius*). This study seeks to determine the potential effects of exposure to a commercial PBDE mixture, DE-71, on the reproductive performance, growth and development of captive American kestrels.

Materials and Methods.

Thirty pairs of adult American kestrels were exposed to an environmentally relevant low dose of 0.3 ppm or a high dose of 1.5 ppm DE-71 added daily to their diet, beginning 30 days prior to pairing and continuing until eggs began to hatch. Control pairs had safflower oil only added to their diet. The DE-71 mixture or safflower-oil only was injected daily into the food item (day-old frozen-thawed cockerel) of the kestrels. Nestlings that were produced by these adults were exposed to the DE-71 through maternal transfer (*in ovo* exposure) only. The first egg of each pair

of birds was collected immediately after it was laid in order to determine concentrations of DE-71 and any potential changes in the egg components (yolk mass, albumen mass, eggshell thickness). Blood was collected from both adult birds and nestlings in order to assess potential effects of DE-71 on circulating hormones, particularly thyroid hormones and growth hormone (juveniles only). Reproductive success was also measured, particularly fertility (the number of fertile eggs compared to eggs laid within a clutch), hatching success (the number of hatchlings versus fertile eggs produced by a pair), and fledging success (the proportion of fledglings to hatchlings per pair). Clutch size (number of eggs laid by a pair), clutch initiation date, egg size parameters and embryonic development were also measured. The growth of nestling birds was measured every five days, with measurements taken of their body mass, the length of their tarsometatarsus leg bone (henceforth referred to as the tarsus), and the length of the ninth primary wing feathers and central rectrix tail feathers. Least square means ± SEMs are presented in this abstract.

Results and Discussion.

Concentrations of DE-71 have been measured in the eggs collected in 2005; results for 2006 are expected in the immediate future. Reflecting the ubiquitous nature of brominated flame retardants, extremely low concentrations of DE-71 were detected in the eggs collected from the control kestrels (11.13 ± 0.90 ng/g ww). Those kestrel pairs in the low-dosed group laid eggs with significantly higher concentrations of DE-71 (1043.46 ± 159.17 ng/g ww) than the controls. Eggs laid by kestrels in the high-dosed group had mean DE-71 concentrations of 4152.30 ± 606.38 ng/g ww.

The reproductive success of the captive kestrels was affected by exposure to the low- and/or high-dose of DE-71. Of the kestrel pairs which produced eggs, the kestrels receiving the high-dose of DE-71 (1.5 ppm) experienced a significant mean delay of 12 days compared to control pairs, from the time the birds were paired until the day that the first egg of their clutch was laid. Kestrels in the low-dose group, that is those receiving 0.3 ppm DE-71, also experienced a delay compared to control pairs, taking 8 days longer than control pairs from the time of pairing to the laying of their first egg. Delays in clutch initiation is cause for concern since such delays may reduce clutch size, hatching and fledging success, in many species of birds.

Consistent with the delays in clutch initiation, the eggs laid by the kestrels exposed to low- or high-doses of DE-71 were significantly smaller than eggs laid by control pairs. The high-dosed pairs laid eggs that were significantly smaller in volume (1054.9 ± 5.3 mm^3) than eggs laid by the low-dosed pairs (1096.9 ± 4.3 mm^3), which in turn, were also significantly smaller than the eggs laid by the control pairs (1129.1 ± 3.7 mm^3). Eggs from the high-dosed birds lost significantly more weight (-0.95 ± -0.03 g) during the first 15 days of incubation, than eggs laid by the control birds (-0.69 ± -0.02 g). In surprising contrast, eggs laid by the low-dosed pairs lost marginally less weight (-0.64 ± -0.02 g) during this time period than the control eggs. These changes in weight loss may be indicative of changes in eggshell porosity and/or thickness.

The reductions in egg size and potential changes in eggshell structure may adversely affect embryonic development and hatching success. Consistent with the kestrels exposed to low- or high-doses of DE-71 laying their clutches later and laying smaller eggs that had differential weight loss during embryonic development, the kestrel pairs exposed to DE-71 also showed reductions in fertility, hatching success, and fledging success compared to the control pairs.

Post-hatch developmental changes were also observed in a sex-specific manner with the kestrel nestlings exposed *in ovo* to DE-71 by maternal transfer. During the 28 day growth period, female nestlings raised by their low-dosed parents were significantly smaller in weight and bone length

compared to the control and high-dosed female nestlings. The female nestlings exposed to high doses of DE-71 generally had longer tarsus bones but were comparable in weight to the control females. However, the start of maximal weight gain was delayed for both the low- and high-dosed female nestlings, and the bone growth rates of the low-dosed females was significantly slower than the control females. In contrast, the male nestlings exposed *in ovo* to low- and high-doses of DE-71 were generally significantly larger in terms of body mass and bone length, although their growth rates were comparable to the control male nestlings. These changes in growth may have the potential to affect survival in wild birds exposed to comparable levels of DE-71. The growth patterns of the low- and high-dosed male nestlings, and the high-dosed female nestlings in this study, are comparable to those observed in nestling kestrels exposed *in ovo* and during the 28-day nestling period to the penta congeners, BDE-47, -99, -100, and -153; PBDE-exposed nestlings of both sexes were heavier and had longer bones and feathers (Fernie *et al.*, 2006) which contrasts with the growth patterns observed in the female nestlings exposed *in ovo* only to low-doses of DE-71. The results of this study suggest that *in ovo* exposure to DE-71 at low, environmentally relevant concentrations are sufficient to adversely affect the growth of nestlings.

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