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Diet exposure to technical hexabromocyclododecane (HBCD) affects testes and circulating testosterone and thyroxine levels in American kestrels (*Falco sparverius*)☆

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ABSTRACT

Hexabromocyclododecane (HBCD) is a high-production-volume, brominated flame-retardant that is used in items such as polystyrene foams. HBCD has been detected in the environment, wildlife tissues and in humans globally with some of the highest recorded levels in predatory birds. This study examined the effects of exposure to environmentally relevant levels of HBCD on the reproductive physiology of captive male American kestrels (*Falco sparverius*), a predatory bird. Two sets of males were used: one group not housed with females (unpaired: $n_c=12$, $n_{HBCD}=10$) and the second group housed with females (breeding: $n_c=10$, $n_{HBCD}=20$). All treatment birds were exposed to 0.51 μg HBCD/g kestrel/day technical HBCD, and controls to safflower oil only, injected into their food during seasonal testicular development. Unpaired males were exposed for 3 weeks and euthanized for testicular analysis. Breeding males were exposed for 3 weeks prior to pairing and throughout the courtship period. The HBCD-exposed unpaired males had heavier testes ($p \leq 0.017$) and a trend towards more seminiferous tubules containing elongated spermatids ($p=0.052$). There was also a moderate increase in plasma testosterone concentrations ($p=0.056$) compared to controls. In breeding males, testosterone levels increased during courtship to culminate in higher levels than controls by the time the first egg was laid ($p=0.010$) and circulating free and total T_4 was reduced throughout. The number of sperm cells reaching the perivitelline layer of the first egg for breeding males did not differ between the two groups. This study is the first report that HBCD exposure at environmentally relevant levels alters reproductive physiology in male birds and suggests that birds may be more sensitive to HBCD than mammals.

1. Introduction

Hexabromocyclododecane (HBCD) is a high production volume, brominated flame retardant (BFR) used in commercial products such as polystyrene foams and textiles, and is one of the major three BFRs in current usage (BSEF, 2010). HBCD is lipophilic and bioaccumulative and has been detected in human and wildlife tissues globally; levels are especially high in species at the top of the food chain such as predatory birds (reviewed in Covaci et al. (2006)). The technical mixture of HBCD consists of three isomers (α-HBCD, β-HBCD and γ-HBCD), but is dominated by γ-HBCD (80%). Consequently, γ-HBCD is found to accumulate in the highest concentrations in abiotic compartments in the environment,

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The experimental protocol was approved by the Animal Care Committee of McGill University. A copy of the approved application was provided with the submission of this article.

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however, α-HBCD predominates in animal tissue (reviewed in Law et al., 2005) including in the eggs of peregrine falcons (*Falco peregrinus*) (e.g., Guerra et al., in press). Recently, temporal increases in HBCD concentration have been recorded in the eggs of peregrine falcons, a terrestrial bird of prey (Johansson et al., 2011), in several wild predatory seabird species (Helgason et al., 2009; Sellström et al., 2003), and in human breast milk (Covaci et al., 2006; Eljarrat et al., 2009; Malarvannan et al., 2009).

The α- and γ-HBCD isomers have been shown *in vitro* to act as antagonists for androgens, estrogen and progesterone by binding to their receptors and all three isomers are moderate to strong thyroid receptor agonists (Hamers et al., 2006). Thus, HBCD has the potential to act as a reproductive endocrine disruptor and exposure may result in some alterations related to gonadal physiology *in vivo*, since both sex steroids and thyroid hormones are important regulators of the reproductive cycle.

The physiological effects of HBCD exposure are still not well understood, including those affecting the male reproductive tract. Adult male rats (F_0) exposed to technical HBCD at high doses (15,000 ppm) had decreased epididymides, reduced sperm counts and lateral head displacement in sperm (Ema et al., 2008). Conversely, F_1-generation rats were developmentally exposed to HBCD via maternal transfer followed by dietary exposure during breeding, demonstrated increased testicular mass, but no differences in sperm number or motility. Also, no alterations in gross testicular histology were noted in comparison with controls (Ema et al., 2008). Levels of thyroid hormone (T_4) and follicle-stimulating hormone (FSH) were reduced in F_0 male rats at high doses of HBCD (15,000 ppm and 1500 ppm, respectively). F_1 males exposed to high dose levels of HBCD (1500 ppm) demonstrated higher levels of 5-α-dihydrotestosterone (DHT), which accompanied the increased testis mass compared to controls (Ema et al., 2008). However, in another study on rats, developmental exposure to technical HBCD resulted in decreased testicular mass without affecting circulating thyroid hormone levels in either sex (van der Ven et al., 2009). The contrasting results of these studies were attributed to the differing rat strains used (Ema et al., 2008). Because effects were only seen at high doses, Ema et al. (2008) postulated that HBCD did not have a major endocrine disrupting effect on sex steroid axes in rats.

Birds are often more sensitive to chemical exposure than mammals (Walker, 1983). In contrast to findings in rats exposed to HBCD (Ema et al., 2008), American kestrel (*Falco sparverius*) pairs exposed daily to technical HBCD (800 ppb) displayed a reduction in some pair-bonding behaviors in both sexes, decreased parental behavior in males (Marteinson, 2011) and smaller eggs laid by females in comparison to controls (Fernie et al., in press; Marteinson, 2011). The timing of breeding was also affected in these kestrels, where pairs exposed to HBCD via their diet initiated egg-laying earlier and produced more eggs than controls but with no augmentation in overall reproductive success (Fernie et al., in press).

Since predatory birds have shown some of the highest recorded levels of HBCD in the wild (Covaci et al., 2006), and appear to have greater reproductive sensitivity to HBCD exposure than rodents or fish (Ema et al., 2008; Kuiper et al., 2007), it is critical to determine the specific effects of HBCD on reproduction at current contamination levels. In the present study, male physiology was examined in captive male American kestrels exposed to HBCD via their diet at a concentration similar to that experienced by wild peregrine falcons (Guerra et al., in press).

2. Methods

The present study used captive-bred American kestrels with documented histories, housed at the Avian Science and Conservation Centre of McGill University. They were exposed to the natural temperature fluctuations and photoperiod only, and had *ad libitum* access to their food source of day-old whole and non-supplemented cockerels (*Gallus domesticus*). The cockerels were obtained from a local hatchery and are sacrificed shortly after hatching and stored frozen. Exposure procedures followed those of previous studies on PBDEs (Fernie et al., 2008) and are described in detail elsewhere (Fernie et al., in press). All exposed birds were treated with 0.51 μg HBCD/g kestrel/day of technical HBCD (HBCD-TM) formulation purchased from Wellington Laboratories (Guelph Ontario), which was dissolved in safflower oil (0.32 μg/μl or 800 ppb) and injected into the brains of the dead cockerel feed daily; control pairs were exposed to the vehicle only (following Fernie et al., in press). The formulation is injected into the cockerel's brain as this is the part that is eaten first by the kestrels, and ensures that most or all of the HBCD-TM is consumed. Injected cockerels were then placed in the enclosures and study subjects fed from them *ad libitum*. Exposure concentrations of HBCD were environmentally relevant and based on the levels recorded in the eggs of wild peregrine falcons (*F. peregrinus*) (Guerra et al., in press). Care of the kestrels conformed to the Canadian Council on Animal Care Guidelines (Olfert et al., 1993) and received approval by the Animal Care Committee of McGill University.

2.1. Study subjects

A set of unpaired males was used for testis evaluation and a second set of breeding males was used to investigate sperm numbers and testosterone levels and a similar timeline for data collection as Marteinson et al. (2011) was employed.

2.1.1. Unpaired males

Ten HBCD-exposed and 12 control males aged 1–9 years old were blood-sampled and euthanized for testes extraction after three weeks of exposure followed by a three-week depuration period. These males were not housed in the presence of females, and are referred to hereafter as unpaired males, as in Marteinson et al. (2011). The HBCD-TM exposure level in these males was determined from a plasma sample taken at the end of the uptake period and another taken at the end of the depuration period (Table 1).

Table 1
Hexabromocyclododecane concentrations (ng/g wet weight)[a] in eggs of breeding pairs and plasma (ng/ml) of unpaired male American kestrels.

	α-HBCD		β-HBCD		γ-HBCD		ΣHBCD	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Breeding pairs (eggs)								
Control eggs	0.3	0.4	0.2	0.1	0.2	0.1	0.6	0.5
HBCD-exposed eggs	163.5	75.1	13.9	5.6	2.6	3.8	179.9	80.6
Unpaired males (plasma)								
End uptake period								
Control	0.12	0.09	0.04	0.05	0.11	0.06	0.26	0.11
HBCD-exposed	4.68	3.20	1.62	1.74	12.19	8.26	18.50	12.97
End depuration period								
Control	0.02	0.02	0.03	0.00	0.03	0.02	0.09	0.04
HBCD-exposed	1.97	0.95	0.06	0.06	0.81	0.6	2.83	1.49

[a] See Letcher et al. (2009) for all details of HBCD isomer concentrations. Breeding males: $n_C=11$, $n_{HBCD}=20$. Unpaired males: $n_C=12$, $n_{HBCD}=10$.