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Effects of Dietary PCB Exposure on Adrenocortical Function in Captive American Kestrels (*Falco sparverius*)

OLIVER P. LOVE,[1,*,†] LAIRD J. SHUTT,[2] JOEL S. SILFIES,[3] GARY R. BORTOLOTTI,[4] JUDIT E. G. SMITS[5] AND DAVID M. BIRD[1]

[1]*Avian Science and Conservation Centre, McGill University, 21,111 Lakeshore Drive, Ste-Anne-de-Bellevue, Quebec, Canada H9X 3V9*

[2]*Environment Canada, Canadian Wildlife Service, National Wildlife Research Centre, 100 Gamelin Blvd., Hull, Quebec, Canada K1A 0H3*

[3]*Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA*

[4]*Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5E2*

[5]*Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 4B3*

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Abstract. We experimentally examined the effects of dietary exposure to polychlorinated biphenyls (PCBs) on adrenocortical function in American kestrels (*Falco sparverius*). Nine captive male American kestrels previously exposed to a PCB mixture (Aroclor^{TM}1248 : 1254 : 1260; 1 : 1 : 1) in their diet were subjected to a standardized capture, handling and restraint protocol designed to produce an increase in circulating corticosterone. A similar protocol has been applied to a wide range of avian species and was used here to evaluate the response of PCB-exposed and control kestrels to a defined physical stressor. Both baseline and stress-induced corticosterone levels were significantly lower in PCB-exposed birds when compared with control birds of the same age. PCB-exposed birds exhibited significantly lower corticosterone levels during the corticosterone response when compared with control birds, independent of body condition. Furthermore, baseline corticosterone concentrations exhibited a hormetic response characterized by an inverted U-shaped dose response in relation to total PCB liver burden. These results support several recent studies which report decreased levels of circulating corticosterone in PCB-exposed wild birds. The results presented here provide the first evidence that exposure to an environmentally relevant level of PCBs (approximately 10 mg/kg body weight) can impair the corticosterone stress response in kestrels, potentially increasing the susceptibility of birds to environmental stressors such as severe weather and predatory and human disturbance.

Keywords: polychlorinated biphenyl; Hypothalamo-Pituitary-Adrenal (HPA) axis; corticosterone; hormesis; American kestrel

*To whom correspondence should be addressed:
Tel.: (604) 291-5422; Fax: (604) 291-3496;
E-mail: olovea@sfu.ca
†Present address: Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada V5A 1S6

Although levels of polychlorinated biphenyls (PCBs) in numerous avian species have declined in the last 25 years, they continue to be the predominant organochlorine contaminant measured in fish-eating bird populations in the Great Lakes region (Bowerman

et al., 1995; Lorenzen et al., 1997; Grasman et al., 1998). Historically, exposure to complex mixtures of contaminants including PCBs has been associated with various aspects of reproductive failure in birds in this region (reviewed in Kubiak et al., 1989; Fox et al., 1991; Gilbertson et al., 1991; Hoffman et al., 1993; Yamashita et al., 1993; Giesy et al., 1994; Ludwig et al., 1995; Hoffman et al., 1998). Studies by Lorenzen et al. (1999) examined the relationship between PCB burdens and the hypothalamo-pituitary-adrenal (HPA) axis in Great Lakes herring gull (*Larus argentatus*) embryos and found that levels of circulating corticosterone were negatively correlated with yolk-sac PCB concentrations.

In the HPA axis, glucocorticosteroids, such as corticosterone, are synthesized and released by the adrenal cortex after stimulation by pituitary-derived adrenocorticotropic hormone (ACTH). This in turn is controlled by corticotropin-releasing factor (CRF), arginine vasotocin (AVT) and mesotocin (MT) released from the hypothalamus as a result of neural input (Romero and Wingfield, 1998). Because of this sequential reaction, glucocorticoid levels increase in response to stress in a delayed fashion (known as the corticosterone response) (Dallman et al., 1987). The HPA axis is considered one of the most important regulatory pathways used by birds to adapt to unpredictable changes in the environment by mediating alternate physiological and behavioral patterns (Silverin, 1998a). One of the potential consequences of HPA axis deregulation by complex mixtures of PCBs is the impairment of the corticosterone response. In birds this response is characterized by elevated plasma corticosterone levels which stimulate behavioral and metabolic responses including increased locomotory activity (Astheimer et al., 1992; Smith et. al., 1994), decreased nocturnal oxygen consumption (Astheimer et al., 1992), lipogenesis (Harvey et al., 1984; Gray et al., 1990), increased food intake (Bray, 1993) and increased energy production from protein catabolism (Gray et al., 1990; Wingfield et al., 1995). The corticosterone response has been shown to vary both daily and seasonally (Wingfield et al., 1992, 1994; Astheimer et al., 1994; Silverin, 1997, 1998a; Silverin et al., 1997), and with the type of environmental stressor (Wingfield et al., 1983; Rogers et al., 1993; Smith et al., 1994; Astheimer et al., 1995; Silverin, 1998b). It may also vary between populations and between individuals of the same species (Wingfield et al., 1992; Astheimer et al., 1994; Schwabl, 1995; Silverin et al., 1997; Silverin and Wingfield, 1998). Body condition is also known to have a significant effect on both baseline and stress-induced levels of corticosterone, with birds in poor condition often exhibiting elevated baseline corticosterone levels and a decreased corticosterone response (Smith et al., 1994; Heath and Dufty, 1998; Kitaysky et al., 1999; Sockman and Schwabl, 2001). Finally, it has been suggested that the length of time a bird spends in captivity and/or the number of times it has been handled may affect the corticosterone response due to habituation to the handling procedure (Dufty Jr. and Belthoff, 1997; Piersma and Ramenofsky, 1998; Cockrem and Silverin, 2002; Love, unpubl. data).

The adrenal cortex, responsible for corticosterone secretion, has been shown to accumulate lipophilic compounds including PCBs in several fish and amphibian species (Colby and Longhurst, 1996; see Hontela, 1997 for review). Effects reported on the adrenal gland associated with PCB exposure include: histological alteration (Bergman and Olsson, 1985), cytotoxic atrophy (Hart et al., 1973) and inhibition of adrenal steroidogenesis (Civen and Brown, 1974). It has also recently been established that adrenal monoxygenases (P-450 family) have the capacity to metabolize xenobiotics, in some cases producing toxic metabolites (Colby and Longhurst, 1996), potentially causing chemically induced lesions. In fact, of all the endocrine tissues reviewed by Ribelin (1984), chemically induced morphological lesions most frequently occurred in the adrenal gland. Several studies have reported the reduced ability of petroleum-dosed birds to deal with the secondary stress of cold temperatures (Holmes et al., 1978, 1979), indicating possible involvement of the corticosterone response system. Recent reports suggest that one of the effects of contaminants on the HPA axis may be the impairment of adrenocortical function (Hontela et al., 1992; Gendron et al., 1997; Hontela, 1997; Lorenzen et al., 1999). The purpose of the present study was to evaluate the effects of dietary exposure to a PCB Aroclor^{TM} mixture on the ability of American kestrels (*Falco sparverius*) to respond to a standardized stressor of capture and handling. The ability of the birds to respond to the stressor was inferred from measurement of immuno-detectable levels of circulating corticosterone. The capture-stress protocol employed to measure this response has been applied to a wide spectrum of species under a number of variable environmental and ecological conditions

(see Schwabl et al., 1991; Wingfield et al., 1992; Wingfield, 1994 for details).

Materials and methods

Study species and experimental design

The study was conducted at the Avian Science and Conservation Centre of McGill University in July 1999 using captive birds aged 1.5–4 years reared from captive-raised adults. Eighteen banded male American kestrels were removed from same-sex communal flight pens and placed singly in outdoor pens measuring $3.5 \times 5.5 \times 3.0$ m ($l \times w \times h$). The 18 males were allowed to acclimate in the pens for 4 days prior to the initiation of the study. The sampling design was completely randomized and a maximum of four birds were sampled per day allowing all 18 birds to be sampled within 5 days. All birds were fed a diet of day-old cockerels *ad libitum* during the sampling. Nine of these males had been fed a diet containing a mixture of PCBs (Aroclor^{TM} 1248 : 1254 : 1260; 1 : 1 : 1, by weight) for 120 days beginning in the spring of 1998 and subsequently a clean diet for 348 days at which time the present study took place. Briefly, the Aroclor^{TM} mixture was dissolved in safflower oil at a concentration of 4.85 mg/g total PCB, which was calculated to provide a dose of total PCBs of approximately 10 mg/kg body weight of kestrel per day (as described in Fernie et al., 2000). A 100 µl aliquot of the dosing mixture was injected intracranially into frozen-thawed day-old cockerels. The cockerels for the control birds received the equivalent intracranial dose of safflower oil only. The dosage levels and the mixture of PCBs were similar to the body burdens reported for small mammals around contaminated sites (see Fernie et al., 2000). The remaining nine control birds were chosen to match the ages of the PCB-exposed birds.

Sampling protocol

Kestrels underwent a standardized capture, handling and restraint procedure known to elicit an increase in circulating corticosterone levels (Wingfield et al., 1983). All testing took place on fasted birds between 10:00 and 13:00 h. Birds were removed from their pens using large scoop nets and a stopwatch was used to record the time of first contact when the pen door was opened. Birds were transported in 8.5 cm diameter opaque tubes closed at one end from the outdoor pens to a holding room.

Individual birds were weighed and approximately 75 µl of whole blood was collected from the left jugular vein upon removal of the bird from its pen (<2 min) using a heparinized 27-gauge needle and 1 ml syringe. Additional blood samples were collected at 5, 10, 30 and 45 min after capture.

Between blood collections, birds were placed individually in wooden opaque holding boxes containing a perch and wood shavings. Individual birds were unable to hear or see other test birds. Unflattened wing chord and tarsus length were measured following the sampling period and combined with mass to provide a measure of body condition. To calculate mass corrected for body size, we first calculated the scores of a principal component analysis (PCA) based on unflattened wing chord and tarsus length of all birds. The scores from the first principal component were used as an estimate of skeletal body size. We then regressed body mass against these PCA scores and used the residuals from this regression as an estimate of mass corrected for body size and included them as a covariate in an analysis of covariance model (ANCOVA). Whole blood samples were centrifuged within 5 min of collection at 10,000 rpm for 10 min. The plasma was removed and stored frozen at $-20\,^{\circ}$C for radioimmunoassay analysis.

Corticosterone radioimmunoassay

Plasma levels of corticosterone were measured using a specific radioimmunoassay (RIA) at the National Wildlife Research Centre, Hull, Quebec. Plasma samples were thawed, vortexed and diluted at a 1 : 10 ratio of plasma to steroid diluent using RIA kits (ICN Biomedicals Inc., Costa Mesa, CA, cat. no. 07-120103) designed for mouse and rat corticosterone analysis (validated in-house for avian plasma). One hundred µl of each sample were added in duplicate to labeled assay tubes. Next 200 µl each of I-125 labeled corticosterone and then corticosterone anti-serum were added to each tube, vortexed and allowed to incubate for 2 h. Then 500 µl of steroid precipitant were added, vortexed and centrifuged for 15 min at 2,300 rpm. The supernatant was removed and analyzed with a Packard Cobra II Auto Gamma-counter (model number

E5002). Each day standard concentrations of corticosterone were used to develop a standard binding curve. Recovery values ranged from 85% to 90% and were used to adjust assayed concentrations of corticosterone. Quality control samples were run with every set and intra- and inter-assay coefficients of variation were 9.4% and 11.6%, respectively.

Contaminant analysis

Congener-specific determination of PCB concentrations in exposed kestrel livers were carried out at the National Wildlife Research Centre according to methods described in Won et al. (2001). Briefly, homogenized tissues were dried with sodium sulfate and extracted with a 1 : 1 mixture of Dichloromethane (DCM) and hexane. Extracts were then cleaned up using gel permeation chromotography and florisil columns to remove high molecular weight biogenic materials and lipids. Organochlorines (including 51 individual PCB congeners) were quantified using a mass selective detector (HP 5970). Levels reported here were corrected for internal standard recoveries which ranged from 85% to 90%.

Statistical treatment

We used a two-factor mixed factorial design, where treatment (either control or PCB) was a between-groups factor and time (1–45 min) was a within-subjects factor or repeated-measure. Initially data were tested for compliance with the assumptions required by a statistical test according to Sokal and Rohlf (1995). At no time were these assumptions violated, and therefore data transformation was not necessary.

To detect differences in corticosterone patterns between the two groups over the 45 min sampling period, a two-way (treatment $\times$ time) repeated-measures analysis of covariance (ANCOVA) was used with treatment and age as factors, body condition as a covariate and serial sampling of the same bird during the stress protocol as a repeated-measure. Linear regression analysis was used to examine the relationship between time after capture and baseline corticosterone concentrations. Baseline corticosterone levels were examined between the two groups using analysis of variance (ANOVA). The relationship between baseline corticosterone and liver PCB burden within the PCB group was analyzed using polynomial regression. Due to the fact that baseline levels were significantly different between PCB-dosed and control groups in this study, Maximal Increase (MI) in plasma corticosterone, as opposed to absolute maximum levels, was used for further statistical comparison. MI levels were calculated by subtracting the baseline corticosterone level for a particular bird from its maximum stress-induced corticosterone level at 30 min and pair-wise *t*-tests were used to compare MI levels between the two treatment groups.

Results

Contaminant levels

Total PCB concentrations of livers from PCB-exposed kestrels in the present study were found to range from 5.4 to 31.5 ppm μg/g (wet wt. basis). Both the class of congeners present and their concentrations in the present study are in agreement with those of the larger group of birds from which they were sampled (Drouillard et al., 2001). Major congeners which were detected in substantial concentrations were: PCB 138, 153, 180 and 187.

Drouillard et al. (2001) also reported the detection of small quantities of PCBs (sum PCB $= 10.3 \pm 3$ ng/ml; mean, SE) in blood plasma of control birds taken prior to dosing on day 0. As noted earlier, these control birds are derived from the same colony as control birds in the present study. The composition of PCBs in controls from Drouillard et al. (2001) consisted of persistent congeners PCB 28, 99, 118, 138, 153 and 180. The authors note that these congeners are typically found in environmental samples and may reflect background contamination of the study birds. However, the levels of PCBs in control plasma were 100–800 fold lower than residues observed in exposed birds at the end of the 348 day elimination period, at the time when the present study was conducted.

Baseline levels of corticosterone

Linear regression analyses showed no effect of time after capture (within 0–2 min interval) on plasma levels of corticosterone in initial blood samples ($r^2 = 0.048$, $F_{1,16} = 0.80$, $p = 0.38$). Thus, initial blood samples were considered to reflect baseline

levels of corticosterone. Baseline levels of corticosterone for the control birds were 2.98 ± 0.50 ng/ml (mean, SE) and ranged from 1.05 to 5.79 ng/ml, whereas those of the PCB-exposed birds were 1.77 ± 0.29 ng/ml (mean, SE) and ranged from 0.69 to 3.6 ng/ml. Baseline levels of corticosterone for the control birds were significantly higher than those of PCB-exposed birds ($t_8 = 3.41$, $p < 0.01$). There was no effect of age ($F_{2,13} = 2.15$, $p = 0.16$) or body condition ($F_{1,13} = 1.93$, $p = 0.28$) on baseline corticosterone levels.

Corticosterone response to stress

All PCB-exposed and control birds responded to the standardized capture and handling procedure by a rapid increase in secretion of corticosterone during the 45 min sampling period (time after capture: repeated-measures ANOVA $F_{4,28} = 19.74$, $p < 0.0001$, Fig. 1). However, the corticosterone response was weaker in PCB-exposed birds than in control birds (treatment effect: $F_{1,13} = 6.75$, $p = 0.02$). In addition, the pattern of corticosterone secretion differed between the PCB-exposed and control groups during the sampling procedure (treatment × time interaction term: $F_{4,52} = 5.82$, $p < 0.001$). There was no effect of age ($F_{2,13} = 2.32$, $p = 0.17$) or body condition ($F_{1,13} = 2.02$, $p = 0.18$) on the corticosterone response. Furthermore, MI corticosterone levels in control birds were 20.37 ± 2.36 ng/ml (mean, SE) and were significantly higher than those of PCB-exposed birds which were 10.53 ± 0.965 ng/ml (mean, SE) ($t_8 = 2.73$, $P = 0.03$).

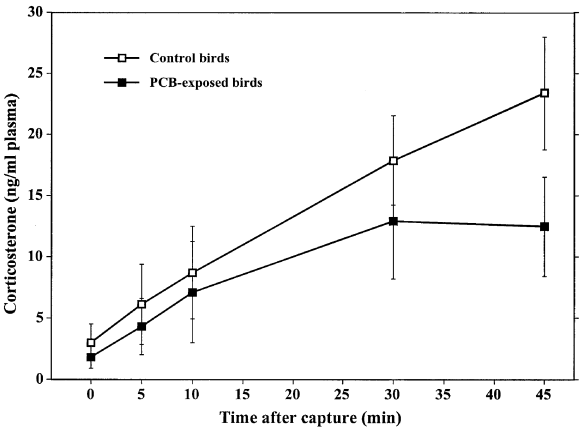

Figure 1. Adrenocortical responses (means, SE) in adult male American kestrels in relation to treatment, either control or PCB-exposed; ($n = 9$ for each group).

PCB burden and circulating levels of corticosterone

Among the PCB-exposed birds, baseline levels of corticosterone were significantly correlated with total PCB burden in an inverted U-shaped dose response (1st order polynomial regression, $r^2 = 0.68$, $F_{2,8} = 6.32$, $p = 0.03$, Fig. 2). There was no such relationship with MI levels of corticosterone either directly (linear regression, $r^2 = 0.05$, $F_{2,8} = 0.37$, $p = 0.56$), or hormetically (1st order polynomial regression, $r^2 = 0.11$, $F_{2,8} = 0.37$, $p = 0.69$).

Discussion

This study relates PCB concentrations in liver tissue to the deregulation of the HPA axis in birds. American kestrels exposed to dietary PCBs had significantly

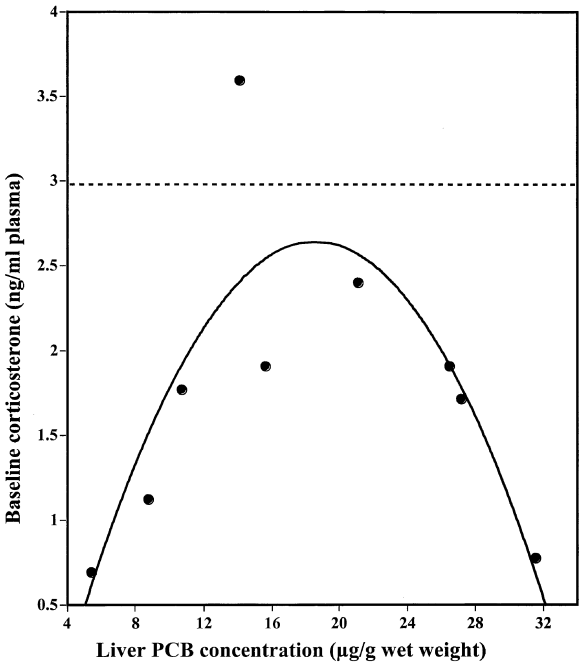

Figure 2. Dose–response curve for PCB-exposed male American kestrels in relation to baseline corticosterone levels. Dashed line represents the average baseline corticosterone level for control birds. Each point represents a single PCB-exposed bird, $n = 9$. $r^2 = 0.68$, $F_{2,8} = 6.32$, $p = 0.03$.

lower corticosterone responses and reached their maximum corticosterone levels earlier than control birds. In addition to significantly lower maximum levels of corticosterone and in agreement with previous studies (Civen et al., 1977; Gorsline and Holmes, 1981, 1982; Lorenzen et al., 1999), circulating baseline levels of corticosterone were also significantly lower in contaminant-exposed animals compared with controls. Furthermore, baseline corticosterone levels in PCB-exposed birds showed an inverted U-shaped dose response relationship in relation to PCB liver burden. Baseline and stress-induced corticosterone levels observed in control birds in the present study are similar to those previously reported for American kestrels (Rehder et al., 1986; Heath and Dufty, 1998). The exposure levels and the mixture of PCBs used in this study were similar to the body burdens reported for small mammals around contaminated sites (see Fernie et al., 2000). Small mammals are known to make up a dominant portion of the diet of wild kestrels (Bird and Palmer, 1988). In addition, PCB egg residues of dosed female mates of the males in the present study were within ranges considered environmentally relevant (Fernie et al., 2000; Fisher et al., 2001). Overall, these data suggest that adrenocortical function in free-living American kestrels may be impaired when exposed to elevated levels of PCBs in the environment.

Implications for wild birds

Although the long-term effects of an impaired adrenocortical function are difficult to assess in captive birds with access to *ad libitum* food and shelter from predation and adverse weather conditions, effects may be significant in wild birds. Due to the stable nature and slow depuration rates of many PCB congeners (Drouillard et al., 2001), wild birds exposed to significant PCB contamination may be unable to elevate corticosterone concentrations to levels required to respond to environmental and physical stressors.

Lorenzen et al. (1999) have suggested that deficits in gluconeogenesis or lipogenic activity may play roles in the wasting observed in young chicks in highly contaminated areas within the Great Lakes basin. Various studies of colonial-nesting waterbirds in the Great Lakes region have documented symptoms associated with wasting syndrome which may be associated with the impairment of corticosterone response systems. These studies include: unexplained weight loss associated with planar halogenated hydrocarbon poisoning (Safe, 1986), elevated chick mortality in certain populations of fish-eating birds in contaminated regions of the Great Lakes (Tillitt et al., 1991), no survival of Caspian tern (*Sterna caspia*) chicks past fledging age collected from a highly contaminated Michigan site (Kurita et al., 1987), and unexplained deaths of emaciated Forster's tern (*Sterna forsteri*) nestlings from Green Bay, with monitored nestlings appearing to gain weight normally until the 14th to 20th day, only to rapidly lose weight until dying (Harris et al., 1993). Recent studies have shown that avian embryos collected from contaminated sites already have lowered corticosterone responses when challenged by a heat stress (A. Lorenzen, unpubl. data). Possible long-term effects may include reduced reproductive output through increased chick mortality, especially since developing young are exposed to many pathogenicand nonpathogenic stresses during development while their corticosterone response systems may only be partially developed (Schwabl, 1999; Love et al., 2003). Furthermore, studies have also shown an association between exposure to organochlorines and impairment of immune responses in Great Lakes fish-eating bird chicks (Grasman et al., 1996) and freshwater fish (reviewed in Hontela, 1997) from contaminated sites. In fact, chronic activation of the HPA axis has been shown to result in the suppression of immune function (Sapolsky et al., 1986; Sapolsky, 1992) and the group of PCB-exposed males from which the birds in the present study were sampled exhibited significantly suppressed antibody production compared with controls (Smits and Bortolotti, 2001). However, at the time of sampling in the present study, PCB-exposed birds exhibited lower baseline and stress-induced corticosterone levels than control birds. It is possible that the PCBs are affecting the immune system independently of the effects of corticosterone. Future studies should examine both the short- and long-term effects of organochlorine contaminants on immune and adrenocortical function concurrently.

Relationship between baseline corticosterone levels and PCB burden

This study found an inverted U-shaped relationship between liver PCB concentration in exposed birds and

their baseline corticosterone levels. This type of dose response is referred to as hormesis (Calabrese and Baldwin, 1999), where at low levels of exposure to contaminants many biological systems display an overcompensation, which results in an apparent low-dose stimulation component to the response curve (Calabrese and Baldwin, 2001a). In the present study, baseline adrenocortical function appears to be affected by concentrations of persistent contaminants in the body. Most studies which discuss hormetic responses involve short-term dosing followed soon after by measurement of a biological endpoint (Calabrese and Baldwin, 2001b). In these cases, values for dosed birds are at least equal to control values, and then increase through stimulation. However, the present study, involving long-term exposure, followed by considerable time for contaminants to have additional effects, produced an apparent hormetic response with baseline corticosterone levels of PCB-exposed birds depressed below those of controls (Fig. 2). As discussed earlier, adrenal monoxygenases (P-450 family) have the capacity to metabolize xenobiotics, in some cases producing toxic metabolites (Colby and Longhurst, 1996), potentially causing chemically induced lesions. The present study may be elucidating long-term damage to the adrenal cortex: remaining intact cells still produce a hormetic baseline corticosterone response in relation to liver PCB concentrations; however because the cortex has been damaged, there are fewer cells overall resulting in baseline levels being depressed below those of control birds. Future dose–response studies should investigate the potential relationship between chronic exposure and impairment of adrenocortical function.

This study has determined that exposure to elevated levels of PCBs is associated with a significant impairment of the corticosterone response system in captive birds. The persistence of PCBs, combined with the sensitivity of the HPA axis to organochlorine insult, suggest that exposure to these contaminants may cause delayed functional deficits later in development or life. Further field studies involving birds from PCB-contaminated sites are required to determine the extent to which the corticosterone response systems of wild birds are affected by exposure to persistent organochlorines, in addition to the effects on lifetime reproductive success. Also, although not investigated during this study, the potential importance of corticosterone binding globulins (CBG) (Deviche et al., 2001) on the concentrations of free corticosterone levels in contaminant-exposed birds remains to be investigated. Finally, dose-dependency should be further investigated in captive birds in order to determine at what levels PCBs affect the avian adrenocortical system. Compromised corticosterone response systems are likely only one consequence of impaired HPA axis function in wild populations, yet this deficit alone can have far-reaching consequences in terms of altered behavioral and metabolic processes necessary for survival.

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