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Adrenal Corticotropin Hormone and Nestling Bald Eagle Corticosterone Levels

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The effects of environmental toxicants on bald eagle (*Haliaeetus leucocephalus*) reproduction are well known for egg hatchability, but not for potential survivability of hatched young. It is known that DDE and crude oil exposure to nestling birds depresses corticosterone levels influencing growth and survivability (Miller *et al.* 1978; Holmes *et al.* 1979; Gorsline and Holmes 1982; Gross 1990). It is also known that administration of adrenal corticotropin hormone (ACTH) to bald eagles causes a marked increase in corticosterone levels in plasma post-injection (Zenoble *et al.* 1985). We used ACTH administration to determine if changes in corticosterone levels were related to plasma concentrations of environmental toxicants, and thus could be used as a biomarker of potential effects on survivability.

MATERIALS AND METHODS

Blood was collected from 19 nestling bald eagles in Michigan in 1997. Sterile techniques were used to collect up to 12 ml of blood from the brachial vein with heparinized syringes fitted with 22 or 24 gauge needles prior to administration of ACTH. Samples of whole blood were transferred to heparinized vacuum tubes (7.5 ml) or to EDTA vacuum tubes (1.5 ml), or vacuum tubes with no additive (1 ml), and kept on ice in coolers. Heparinized tubes were used for organochlorine pesticide and PCB analysis. EDTA tubes were used for corticosterone analysis. Tubes with no additive were used for analysis of metals including mercury. After initial venipuncture, 0.125 mg ACTH (Cortosyn, Organon, Inc., W. Orange, NJ) was administered intramuscularly into the pectoral muscle. After 30 min a second venipuncture occurred and 1.5 ml of blood was collected and transferred to a second EDTA vacuum tube. The blood in the heparinized and EDTA tubes were centrifuged within 45 min of collection. Tubes with no additive were not centrifuged and were frozen without treatment. Blood plasma was decanted and transferred to vacuum tubes. All vacuum tubes were then frozen. We determined the age and sex of each nestling by measuring the 8th primary feather and foot pad of nestlings and comparing them to mathematical growth rate and sexual dimorphism equations (Bortolotti 1984).

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Concentrations of total PCBs and organochlorine pesticides were determined for all plasma samples collected. Gas chromotagraphy was performed on a Perkin Elmer 8500 gas chromatograph, with a ^{63}Ni electron capture detector, and a fused silica capillary column DB-5 (J&W Scientific, Folsom, CA), 30 m x 0.24 mm i.d., 0.25 μm film thickness, using methods previously reported (Mora *et al.* 1993). Whole blood was analyzed for mercury by the Animal Health Diagnostic Laboratory at Michigan State University using methods previously reported using the cold vapor technique (Evers *et al.* 1998).

Corticosterone was measured in plasma using a commercially available radioimmunoassay kit (COAT-A-Count Rat Corticosterone, Diagnostic Products Corp., Los Angeles, CA). The assay was performed in accordance with the protocol provided by the manufacturer of the kit. Use of this assay for measurement of corticosterone in eagle plasma has been previously reported (Zenoble *et al.* 1985).

Regression analysis was utilized to establish the impact of environmental toxicants (i.e., PCBs, DDE, or mercury levels) and breeding area location (i.e., Interior locations or Great Lakes locations, those within 8 km of the Great Lakes) on corticosterone difference (i.e., the difference between pre- and post-injection corticosterone levels). Three separate regression models were defined to accomplish this. In each one, corticosterone difference was the dependent variable and either PCBs, DDE, or mercury was one independent variable and location was a second independent variable. Due to previously reported severe anemia in one nestling due to infestation of *Leucocytozoon toddi*, this breeding area was not utilized in our analyses (Stuht *et al.* 1999). All calculations were performed using the General Linear Model procedure of SAS (SAS Inc. 1991).

RESULTS AND DISCUSSION

Concentrations of Total PCBs, *p,p'*-DDE, mercury and corticosterone varied among breeding areas (Table 1). We did not find any differences in the concentrations of these compounds among sex or age of nestling.

Results of the regression analysis indicated 1) a relationship between corticosterone difference and the combination of DDE and location ($p=0.0098$, $R^2=0.6857$) with both independent variables significant (DDE $p=0.0370$, location $p=0.0179$; Fig. 1a), 2) a relationship between corticosterone difference and the combination of PCBs and location ($p=0.0134$ $R^2=0.6597$) with both independent variables significant (PCBs $p=0.0528$, location $p=0.0115$; Fig. 1b), and 3) no relationships between corticosterone difference and the combination of mercury and location. The difference in corticosterone concentrations increased as concentrations of both DDE and PCBs increased for both Interior and Great Lakes breeding areas. Interior breeding areas, however, had a much greater increase than Great Lakes breeding areas. As discussed below, the lesser increase for Great Lakes breeding areas may have some negative effects for survival.

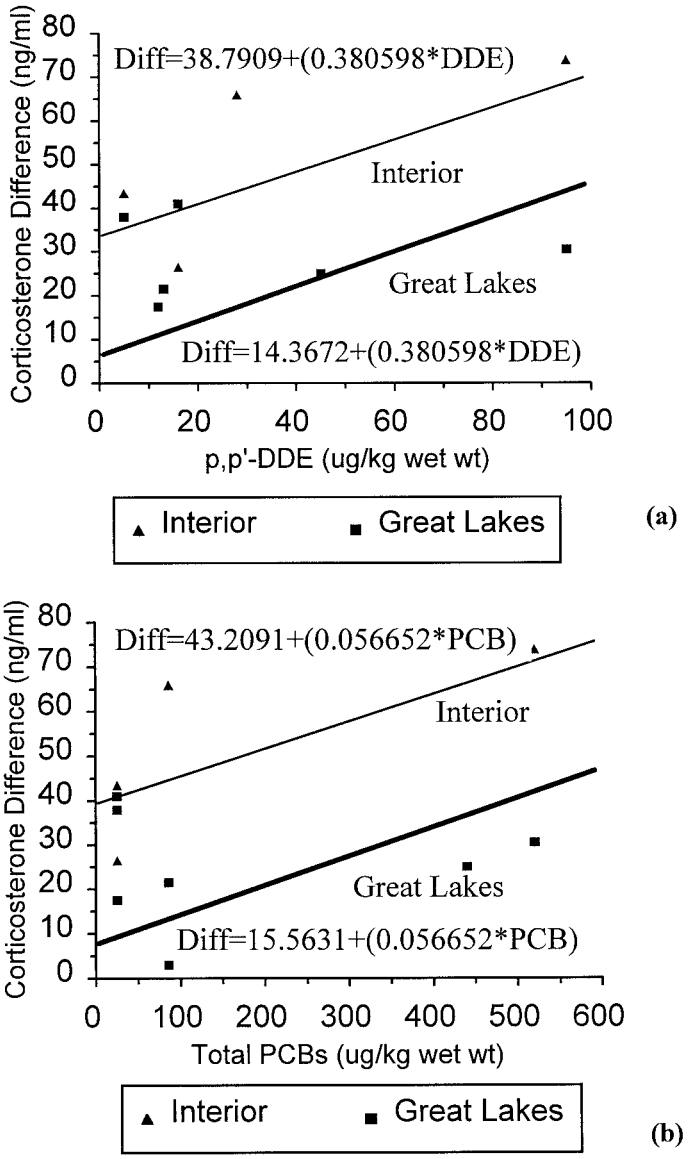

Figure 1. Relationships between differences in plasma corticosterone levels (ng/ml) pre- and post-injection of 0.125 mg ACTH and plasma concentrations of **(a)** *p,p'*-DDE (μg/kg) (Model: p=0.0098, R^2=0.6856) and **(b)** Total PCBs (μg/kg) (Model: p=0.0134, R^2=0.6597) in nestling bald eagles from both interior and Great Lakes breeding areas.

Table 1. Location, concentrations of Total PCBs, *p,p'*-DDE in plasma, mercury in whole blood, and plasma corticosterone pre- and post-injection of 0.125 mg ACTH, for nestling bald eagles from Michigan.

Breeding Area	Interior or Great Lakes Breeding Area	Total PCBs (μg/kg, wet wt.)	*p,p'*-DDE (μg/kg, wet wt.)	Hg (μg/kg, wet wt.)	Corticosterone (ng/ml)	
					Time 0 min	Time 30 min
Ag02	Interior	25	12	310	49	70
Ag02	Interior	25	20	320	40	72
By01	Great Lakes	72	13	30	121	189
By01	Great Lakes	100	13	30	119	133
Cp02	Great Lakes	25	12	210	61	64
De17	Great Lakes	640	46	150	82	113
De17	Great Lakes	240	44	140	72	102
Mc22	Great Lakes	300	86	420	42	77
Mc22	Great Lakes	180	41	480	49	90
Mo04	Great Lakes	140	24	30	181	152
Mo04	Great Lakes	200	24	30	39	111
Ne04	Interior	86	28	120	70	136
Sc04	Interior	32	90	260	37	24
Sc07	Interior	520	95	240	43	117
Sc12	Interior	25	5	240	84	112
Sc12	Interior	25	5	230	81	140
Sg01	Great Lakes	25	10	90	92	105
Sg01	Great Lakes	58	10	160	62	84
Sg02	Great Lakes	190	32	160	110	135

Previous studies have found increases of corticosterone in chickens and ducks after handling but not to the same degree as increases induced by administration of ACTH (Beuving and Vonder 1978; Harvey *et al.* 1980). Different studies have found species differences in response to corticosterone increases after ACTH administration and differences due to age and sex of test animal (Beuvin and Vonder 1978; Walsh *et al.* 1985; Zenoble *et al.* 1985; Davis and Siopes 1987). Care must be made in interpretation of these preliminary results.

We found no differences related to handling stress, nor were there relationships between corticosterone concentrations at Time 0 min and any of our three contaminants of interest, DDE, PCBs, or mercury.

Since corticosteroids in birds function to control salt balance and some metabolic activities, alteration of their normal levels can potentially affect survival.

Corticosteroids may have immunosuppressive activities in birds (Rattner and Eastin 1981) and assist in the regulation of fat deposition in birds. For migratory birds and those that over-winter in cold climates, alteration of fat deposition could be related to affect survival due to greater susceptibility to cold stress (Holmes *et al.* 1978; Holmes *et al.* 1979). Also, since bioaccumulative compounds are lipophilic, this may play a role in altering circulatory levels of these compounds.

Our preliminary data suggest that exposure to environmentally derived organochlorines in early development of bald eagles is, on a regional level (i.e., Great Lakes or Interior breeding area) associated with lesser induction of plasma corticosterone when induced with ACTH. Increases in corticosterone induction were, however, positively related to increases in either DDE or PCBs. The overall lesser induction of the Great Lakes breeding areas may have implications for long term survivability. This technique shows some promise for development as a biomarker for bald eagles. Further investigations, both in the laboratory and under field conditions, are necessary to validate this technique.

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