Environmental Endocrine Disruption in Avian Wildlife

Project Scope

The lipophilic nature of many putative endocrine disrupting chemicals (EDCs), including several halogenated aromatic hydrocarbons (HAHs), promotes their bioaccumulation in food chains. In mammals, exposure to certain HAHs interferes with normal development by activating the cytosolic phosphorylation transduction pathways by which endogenous steroid hormones and growth factors communicate their signals to the cell nucleus. Thus, HAHs are considered prime candidates for causing the reproductive failures and developmental defects observed in other vertebrates (i.e., birds, reptiles, and amphibians) in HAH-contaminated areas of North America and elsewhere. The failure of experiments with laboratory rodents exposed to HAHs to replicate those effects might result from taxonomic differences between mammals and the other classes of vertebrates in one or more of the following factors: mode of action of HAHs, relative sensitivity to HAHs, or interactions between HAHs and endogenous factors that control cell function and differentiation

Grant Title and Principal Investigator

Environmental Endocrine Disruption in Avian Wildlife

Bill L. Lasley, University of California – Davis

EPA STAR Grant #R826298

Key Findings and Implications

- A series of experiments demonstrated that female chickens are as sensitive as males to adverse effects from TCDD exposure, and exogenous estrogen administration did not protect birds against those effects, in contrast the findings in mammalian models.
- Estrogen treatment of male birds resulted in qualitatively similar lipid profiles to mature laying hens and reduced secondary male characteristics (i.e., comb size).
- TCDD antagonized several of the feminizing effects of exogenous estrogen administration in male chickens.
- Specific adverse effects of dioxin on key parameters of lipid mobilization may explain the wide range of developmental defects that are observed in egg-laying species exposed to certain halogenated aromatic hyrdrocarbons (HAHs) compared with placental mammals.

Project Period: February 1998 to January 2001

Mammalian sensitivity to HAHs is sex-specific, owing to differences in the mechanisms of toxicity in males and females at the cellular level. The differences in sensitivity are evident at the organismal level in, for example, a greater loss of body weight in male than in female rodents exposed to the same dose of dioxin; a difference that is reduced by administration of estrogen to males.

It is not known whether birds exhibit similar gender differences in sensitivity. Because birds use stored lipids for migration and reproduction to a greater extent than do mammals, birds might be more susceptible to HAHs released from their lipid reserves during these times. In addition, the physiological changes associated with lipid synthesis and metabolism necessary for egg production might render female birds particularly sensitive to HAH exposures. Thus, the main objectives of this research were to:

1. Characterize the mechanisms of HAH toxicity in males and females of a model avian species, the domestic chicken;
2. Determine the role of altered of sex hormone signaling in the toxicity of HAHs; and
3. Develop evidence that disruption of growth factor and hormone signals interferes with sexual differentiation in avian species.

A series of experiment were conducted to address these objectives. *In vivo* toxicity studies were carried out in 7-9 week-old chickens (*Gallus domesticus*), and *in vitro* studies were performed using abdominal adipose and liver tissue preparations from the treated chickens. The HAH 2,3,7,8-tetrachlorodibenzo-p-

dioxin (TCDD) was selected as a model EDC based on previous studies with mammals that demonstrated interactions of TCDD with sex steroids and a protective effect of estrogen against the adverse effects of TCDD *in vivo* and *in vitro*.

Project Results and Implications

Interactions of estrogen and TCDD

Embryos of chickens were treated with TCDD in combination with sex steroids to determine the effects of HAHs on sexual differentiation and lipid metabolism. Estrogen treatment of male birds resulted in qualitatively similar lipid profiles to mature laying hens and estrogen-treated immature hens, thus providing a model by which to study dioxin-estrogen effects on lipid metabolism in the absence of the energetic needs of actual egg production. Injection of estrogen in males (1 mg/kg bw-day for 3 consecutive days) decreased comb height (24 percent) and comb length (26 percent) (see Figure 1). It also resulted in decreased adipose tissue (AT) lipoprotein lipase (LPL) activity (assessed *in vitro*) relative to AT mass (51 percent), while the ratio of liver to body weight was increased by 14 percent and body weight gain was increased by 28 percent compared with controls. TCDD administered at 0.05 mg/kg-day resulted in reduced comb length (17 percent) but not reduced comb height (see Figure 1). TCDD treatment also resulted in a substantially reduced AT LPL activity indexed to AT mass (70 percent) and increased ratio of liver to body weight (54 percent) compared with controls (see Table 1). TCDD treatment (alone and in combination with estrogen) did not significantly affect body weight compared with the control group. The combined treatment with 1 mg/kg-day estrogen and 0.05 mg/kg-day TCDD produced combs with height and length intermediate to those produced by treatment with estrogen or TCDD alone (see Figure 1), indicating an antagonistic effect of TCDD on the feminization of the male comb induced by injection of estrogen. The combined treatment also resulted in 30 percent larger livers relative to body mass and a 37 percent reduction in body weight gain compared with the estrogen-alone treatment, but the combined estrogen/dioxin treatment was not significantly different from the dioxin-alone treatment. These data show that estrogen did not protect against TCDD toxicity as evidenced by the measures used here (see Table 1).

Table 1. Physical alterations in immature male chickens treated with estrogen (1 mg/kg-day) and dioxin (0.05 mg/kg-day) alone or in combination

	Vehicle Control	TCDD alone		E2 alone		E2+TCDD	
	Mean±SD	Mean±SD	% Control	Mean±SD	% Control	Mean±SD	% Control
Comb							
Height (cm)	2.22±0.34 (a)	2.10±0.35 (a)	95	1.68±0.30 (b)	76	1.72±0.19 (ab)	77
Height index (cm/kg)	1.40±0.28 (a)	1.26±0.24 (a)	90	0.940±0.1 (b)	67	1.13±0.07 (ab)	81
Length (cm)	5.90±0.84 (a)	4.88±0.97 (b)	83	4.38±0.50 (b)	74	4.16±0.34 (b)	71
Length index (cm/kg)	3.69±0.39 (a)	2.91±0.47 (b)	79	2.46±0.21 (b)	67	2.75±0.31 (b)	75
Body/Tissue							
Body weight gain (kg)	0.296±0.079 (a)	0.268±0.067 (a)	91	0.379±0.061 (b)	128	0.239±0.039 (a)	81
Liver weight (g)	35.5±2.8 (a)	57.4±8.2 (c)	162	45.4±7.2 (b)	128	50.1±5.8 (bc)	141
Liver weight index (g/kg)	22.2±1.4 (a)	34.2±2.3 (c)	154	25.3±1.2 (b)	114	32.8±1.3(c)	148
AT weight (g)	8.5±4.1 (a)	16.7±10.9 (a)	197	21.4±17.3 (a)	253	16.3±6.0 (a)	192
AT weight index (g/kg)	5.27±2.30 (a)	9.56±4.90 (a)	181	11.4±7.72 (a)	216	10.9±4.63 (a)	207

(a,b,c): Treatment means within the same row not sharing a common letter are statistically different (Tukey's test, $P < 0.05$).
Abbreviations: AT = adipose tissue; SD = standard deviation; E2 = estradiol; TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Experiments on immature male chickens injected with TCDD or estrogen alone or in combination revealed different effects and interactions on lipid concentrations in plasma and the liver. For example, treatment with estrogen alone increased hepatic total triacylglyceride concentrations compared with controls, whereas treatment with TCDD alone did not. Treatment with both estrogen and TCDD revealed that TCDD antagonized the hepatic triacylglyceride effects of treatment with estrogen (i.e., there were no differences from the controls). In plasma, however, TCDD alone stimulated increases in total lipids and total triacylglycerides compared with the controls. Treatment with estrogen alone and TCDD in combination with estrogen resulted in no changes in measured plasma lipids compared with the controls.

Another example of the antagonistic effect of TCDD on estrogen-induced changes in lipid status was seen in effects on concentrations of the fatty acid 22:6n3 in plasma and liver. This fatty acid is particularly important because of its prevalence in the brain, because avian embryos accumulate it in the brain and retina; and because changes in n-3 fatty acids of laying hens alters 22:6n3 concentrations in yolk and in embryonic brain and retina. Immature male chickens treated with estrogen alone exhibited increased plasma and liver concentrations of 22:6n3 compared with the controls, while 22:6n3 concentrations were unchanged in birds treated with estrogen plus TCDD or TCDD alone.

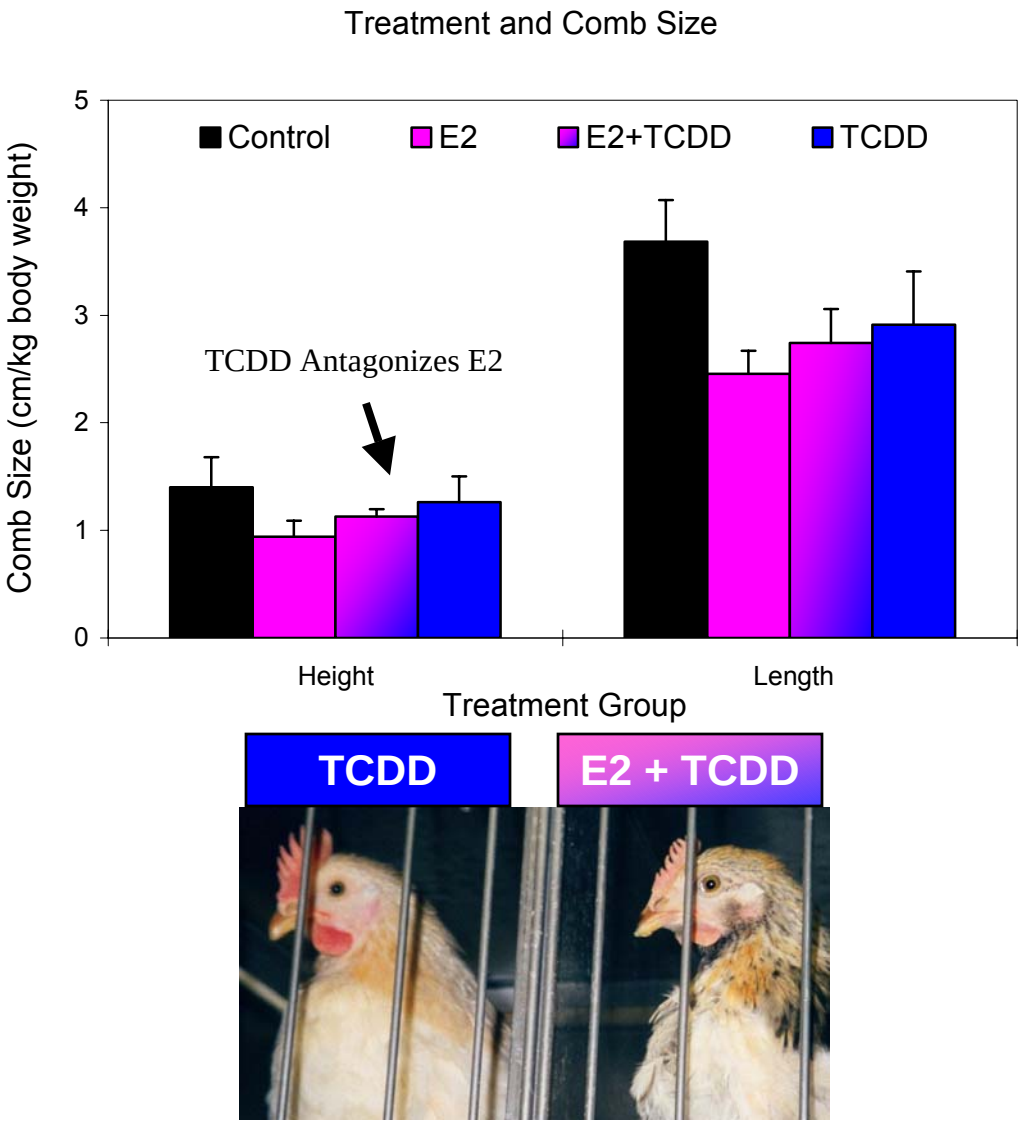

Figure 1. Effects of dioxin and estrogen treatment on comb size in male chickens.

These experiments indicate that the nature of the interaction between dioxin and estrogen in birds differs from that found in mammals. The protective effect of estrogen against TCDD toxicity that has been observed in mammals was not found in the chicken model. The antagonism of several estrogen effects by TCDD in the male chicken model indicates its effect as an EDC in birds. The effects of TCDD on baseline and estrogen-induced changes in several key measures of lipid metabolism may help to explain the wide range of developmental defects that are observed in egg-laying species compared to placental mammals.

TCDD-induced wasting syndrome

TCDD induces a wasting syndrome in mammals that is associated with changes in liver and adipose tissue. Among these changes are decreased AT LPL activity and glucose transporting (GT) activity, with differential sensitivity between genders. To extend these findings to an avian model, studies were

performed to characterize the effects of TCDD in 7-9 week old male and female chickens following single i.p. doses of 0.010 and 0.10 mg TCDD/kg bw, and observing them 10 days after treatment. These doses were selected in order to ensure that clear adverse effects would be seen in both genders and are within range of TCDD doses used previously to elicit overt toxicity in young chickens. This study demonstrated significant TCDD-inducing body wasting in both males and females. In vitro analysis demonstrated that TCDD also resulted in the induction (though not in a dose-response manner) of CYP1A enzymes in both sexes, confirming previous studies, and decreased LPL and GT activities, as have been reported in mammals. Induction of CYP1A is a biomarker of exposure often used in risk assessments for dioxin and dioxin-like chemicals. In contrast to mammals, however, the decreased LPL activity in the chickens was not associated with a decrease in adipose tissue mass. The data from this study underscores the importance of vertebrate class differences in the responses of males and females to EDCs.

Table 2. Alterations in chicken body weight by gender 10 days after TCDD treatment

	Body Weight (g) ± SD			
	Initial	Post-treatment	BW Gain	% BW Gain
Males				
Vehicle Control	836±132	1132±150	295±29 (a,b,c)	35.3
10 µg TCDD /kg	728±143	934±137	206±17 (a,b,d)	28.3
100 µg TCDD /kg	924±143	1098±137	173±7 (a,c,d)	18.7
Females				
Vehicle Control	772±177	958±186	186±25 (a,b,c)	24.1
10 µg TCDD /kg	758±88	909±107	151±25 (a,b,d)	19.9
100 µg TCDD /kg	712±76	883±145	97±51 (a,c,d)	12.2

(a) Significantly different in the mean values among the treatment groups (one-way ANOVA, $P < 0.05$).
(b) Control vs. 10 µg TCDD /kg.
(c) Control vs. 100 µg TCDD /kg.
(d) 10 µg TCDD /kg vs. 100 µg TCDD /kg

Taken together, these studies suggest that potent HAHs and estrogen act as modulators for changes in body weight, energy homeostasis, and lipid metabolism in avian species. The mechanisms by which estrogen and dioxin interact with hepatic lipid synthesis and metabolism appear to differ between birds and mammals.

Investigators

Bill L. Lasley – University of California, Davis
Alan J. Conley – University of California, Davis
Essam E. Enan – University of California, Davis
Michael D. Fry – University of California, Davis
James W. Overstreet – University of California, Davis

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