

**EFFECTS OF AROCLOR 1254 ON ENDOCRINE PHYSIOLOGY  
IN THE CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)**

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**EXTENDED ABSTRACT ONLY – DO NOT CITE**

**Introduction**

The channel catfish, *Ictalurus punctatus*, has a wide distribution in North America. As an obligate benthic species, catfish are frequently exposed to pollutants. Concern exists that these pollutants may affect individual traits and consequently population stability. Recent studies have demonstrated that many pollutants, called endocrine disrupting chemicals (EDCs), can negatively effect how teleosts adapt to changes in their environment (e.g., salinity) (Fairchild *et al.*, 1999; Madsen *et al.*, 1997). These findings underscore the necessity for understanding how EDC exposure influences individual traits, particularly endocrine physiology.

To examine this, a better understanding of the roles that the pituitary hormones, growth hormone (GH) and prolactin (PRL), play in the environmental physiology of the channel catfish are needed. To address this, we have established a method to measure blood levels of GH in channel catfish. We report, herein, the effects of polychlorinated biphenyls (Aroclor 1254) on plasma GH levels in catfish. Our aim is to understand how pituitary hormones influence adaptive responses of catfish to EDC(s) and, in turn, how EDCs influence endocrine physiology.

## **Methods**

### *Assay Development:*

For the GH ELISA, a 96-well plate was coated with 250ng/ml catfish GH in carbonate buffer, with additional wells for non-specific binding (NSB), and was incubated overnight at 4°C without shaking. Wells were then emptied and blocked (without shaking) with PBST-NGS (5%) (Normal Goat Serum) overnight at 4°C.

Hormone standards and plasma samples were serially diluted in PBST-BSA (1%) and mixed 1:1 and 1:4, respectively, with the primary antibody diluted 1:10,000 in PBST-NGS (2%). Controls to determine maximum and non-specific binding were also prepared. Tubes were incubated at 37°C for ninety minutes, at which time 100µl of each reaction was pipetted into the 96-well plate and incubated at 37°C for ninety minutes. The plate was then washed, coated with secondary antibody conjugate (GAR-HRP: goat anti-rabbit horseradish peroxidase) diluted 1:5,000 in PBST-NGS (2%) and incubated at 37°C for ninety minutes. Wells were washed, developed (10 minutes) by the addition of o-phenylenediamine dihydrochloride and H<sub>2</sub>O<sub>2</sub> (substrate) and read at 450 nm using a Molecular Devices plate reader.

### *In Vivo Studies:*

Animals were held in dechlorinated fresh water. Anesthetized animals were given i.p. injections of vehicle (corn oil) or vehicle containing estradiol (5 mg/kg) or the PCB mixture Aroclor 1254 at 1, 10 and 100 mg/kg. Seven days after injection, the animals were sampled for plasma and other tissues for analytical work.

## Results

Using optimized conditions (Ab dilution of 1:10,000 and coating antigen of 250ng/well), we have determined the best standard concentration range, for our ELISA, to be 0.58 ng/ml to 150 ng/ml (Figure 1).

To demonstrate GH-Ab specificity, dilutions of pituitary homogenates and purified hormones from catfish (PRL) and other teleosts were tested. Catfish pituitary homogenate dilutions were parallel to our standard curves, whereas pituitary homogenates or purified hormones (data not shown) from other teleosts were not. A 1:4 plasma dilution was found to be within the linear range of the plasma binding curve and was used for all assays.

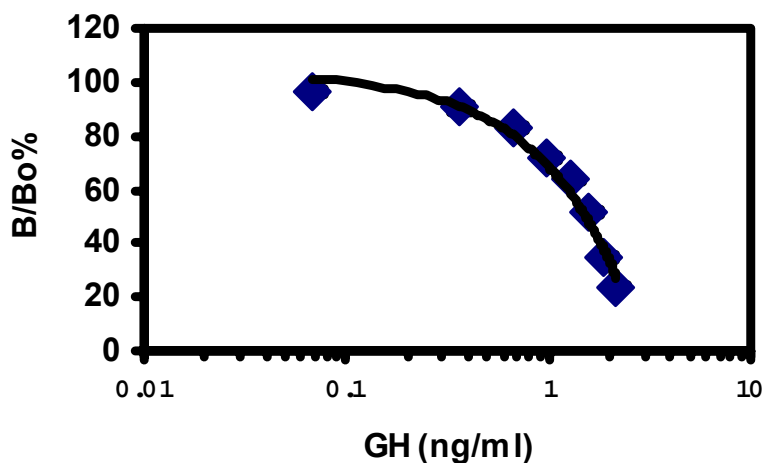


Figure 1: Standard curve of native channel catfish growth hormone (GH) in a competitive ELISA. The ratio of the quantity bound (B) to the maximum binding (Bo) (zero or lowest standard) is determined by optical density (O.D.) at 450 nm and is shown as a percentage for GH standards ranging from 1.17 ng/ml to 150 ng/ml. All values are means + SEM

To assess whether the PCB mixture, Aroclor 1254, exerted any estrogen-like actions in the channel catfish, animals were injected with oil implants containing estradiol or Aroclor 1254 at different doses. Estradiol significantly ( $P < 0.05$ ) elevated plasma GH levels above control values (Figure 2). While mean GH levels in the Aroclor-treated groups were lower than control values, only the 10 mg/kg dose group was significantly ( $P < 0.05$ ) lower than the control group.

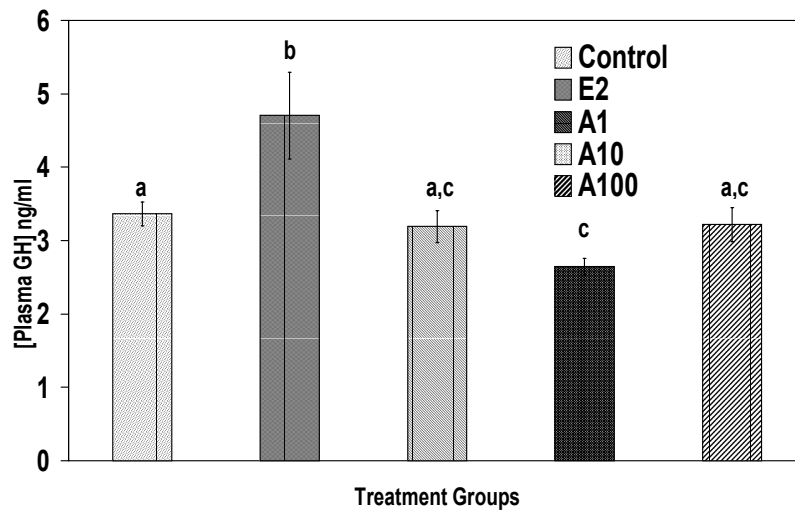


Figure 2: Effects of estradiol and Aroclor 1254 (PCB) injection on plasma GH levels in channel catfish. Plasma GH levels were determined using a homologous ELISA. All values are means  $\pm$  SEM. E<sub>2</sub>= 5 mg/kg Estradiol, A<sub>1</sub>=1 mg/kg Aroclor, A<sub>10</sub>=10 mg/kg Aroclor, A<sub>100</sub>=100 mg/kg Aroclor.

### Discussion

We have developed an ELISA for the measurement of GH levels in channel catfish, thus enabling investigations into how environmental stressors alter levels of this hormone in this species.

Our results show that estrogen stimulates GH secretion in the channel catfish which is consistent with published findings in goldfish (Zou *et al.*, 1997).

Recent studies have reported that EDCs can influence pituitary CYP1A1 and -1A2 expression suggesting that pituitary P450 expression may influence pituitary physiology. In support of this, Cravedi and co-workers (Cravedi *et al.*, 1995) demonstrated that GH treatment reduced induction of hepatic xenobiotic metabolizing enzymes in trout, suggesting the presence of an intricate regulatory relationship between the pituitary and hepatic physiology in response to EDC exposure. Therefore, it would seem reasonable to propose that a reduction in plasma GH, following exposure to a xenobiotic, has adaptive significance in that such a reduction would facilitate xenobiotic metabolism by P450 enzymes in the liver. Additional work is underway to examine the effects of Aroclor 1254 on other metabolic and endocrine sectors.

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