

# **Report for 2002MS2B: Screening of Environmental Contaminants Detected in Mississippi Sediments as Inducers and/or Inhibitors of CYP1B1 Expression in Channel Catfish - Continuation**

- Conference Proceedings:
  - Metzger, Christine U., and Kristine L. Willett, 2002, Environmental contaminants that affect CYP1B gene expression in channel catfish (*Ictalurus punctatus*). Proceedings of the 32nd Mississippi Water Resources Conference, Mississippi Water Resources Research Institute, Mississippi State, MS, page 126 (Abstract)
- Other Publications:
  - Butala, H., Metzger, C., Rimoldi, J., and Willett, K.L. (submitted) 2003, Microsomal estrogen metabolism in channel catfish. Marine Environmental Research.

**Report Follows:**

## PROBLEMS AND RESEARCH OBJECTIVES:

This project is specifically aimed at characterizing the utility of a recently discovered cytochrome, CYP1B1, as a marker of exposure to contaminants that have been reported by the USGS NAWQA and BEST programs in Mississippi sediments and fish samples. Because channel catfish (*Ictalurus punctatus*) are such an abundant and economically significant species in Mississippi, they are being used as the test organism in these studies.

## METHODOLOGY:

There are three aspects of our **Laboratory Studies** ongoing.

**1) Cloning:** Last quarter we had dosed an additional channel catfish with 20 mg/kg BaP for 4 days in order to get a large supply of total RNA and ultimately mRNA in order to try a new 5' RACE cloning protocol. This mRNA was reverse transcribed with a new high temperature protocol using both universal and gene specific primers. 5'RACE PCRs are ongoing to clone the full length sequence.

**2) Quantitative Real-Time RT PCR (qRT/RT-PCR):** This quarter we have several difficulties (cold weather, building maintenance etc) which affected our catfish culture. We lost several animals, and we had to put the remaining animals on an antibiotic treatment. Because we do not want to use fish until they return to normal physiological conditions, we have not done any primary cell culture experiments this quarter. The catfish, though are now healthy again and we can resume our experiments exposing liver and gill cells to PCB 77, PCB 126, PCB 153, p,p'-DDT, and TCDD.

**3) Estrogen metabolism in control and benzo(a)pyrene exposed and wild channel catfish:** All samples have now been assayed for their ability to metabolize estrogen. Mammalian CYP1B1 is involved in metabolism of estradiol to 4-hydroxyestradiol whereas CYP1A1 forms predominantly the 2-hydroxyestradiol. Microsomes of liver, gill and gonad tissues of control, dosed, and Delta catfish (gill and liver only) were made. These samples were incubated for two hours with estradiol, and the estrogen metabolites formed were quantitated by a GC-MS method that we have optimized. The liver microsomal formation of 2- and 4-hydroxyestradiol are shown in Figure 1. Bars with the same symbol are not significantly different from each other ( $p < 0.05$  by ANOVA). In the liver microsomes, BaP treatment induced formation of both the 2- and 4-hydroxyestradiol metabolites relative to control laboratory fish. The amount of 2-hydroxyestradiol metabolite formed by Lake Roebuck liver microsomes was also statistically higher compared to controls. In contrast, the 2-hydroxyestradiol formed by Bee Lake and Sunflower River fish liver microsomes was statistically lower than laboratory controls. Like the 2-hydroxyestradiol metabolite, 4-hydroxyestradiol was induced in the BaP exposed samples, but the trends in 4-hydroxyestradiol formation in Delta fish did not follow the same trends as the 2-hydroxyestradiol formation. The gill microsomes (Figure 2) had far less estrogen metabolic capability than either the liver or gonad microsomes, and only 2-hydroxyestradiol was detected following gill incubations. Furthermore, there were significant differences between all five gill microsome preparations in their ability to form the 2-hydroxyestradiol metabolite. In gonad microsomes there was no significant difference between laboratory control and BaP-dosed fish. Because the testis is a much smaller tissue, we were unable to do multiple replicates, and the protein concentrations were much lower. Therefore, there was higher variability in metabolite production by gonad microsomes compared to the other two tissues. Future experiments are going to use a CYP1A antibody in the incubation. It is hoped that the antibody will knock out any CYP1A enzyme activity, and in this way we will be able to determine the relative contribution of CYP1A in the metabolism of estradiol (and hence hypothesize on the role of CYP1B).

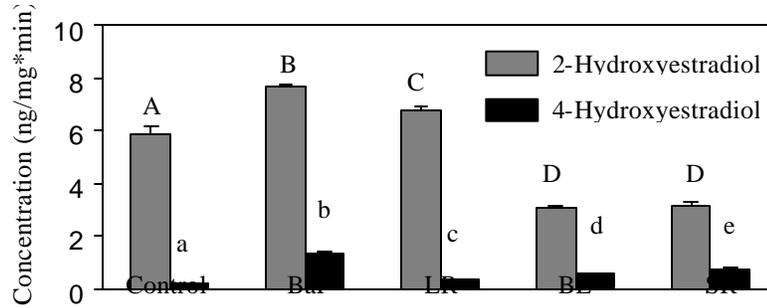


Figure 1

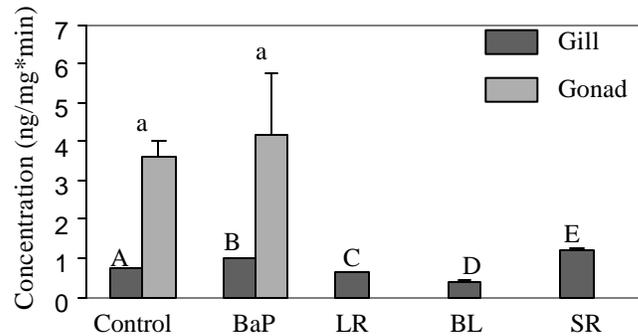


Figure 2

**SIGNIFICANCE/PRESENTATIONS:**

Overall, these results aim to characterize utility of CYP1B1 as a biomarker of exposure to environmental contaminants in channel catfish collected from Mississippi lakes and rivers. As mentioned, we will continue to compare the results from laboratory animals to those collected from Mississippi waterways.

These results “Microsomal Estrogen Metabolism in Channel Catfish” were submitted for presentation at the Pollutant Responses in Marine Organisms Meeting (May 9-13<sup>th</sup>, 2003, Tampa FL), and we are currently writing the results in paper form to be submitted for publication in *Marine Environmental Research*.