

Poster #14

Androstenedione is Present in a River Containing Paper Mill Effluent and Masculinized Fish

Ronald Jenkins¹, Robert Angus², Heather McNatt², W. Mike Howell¹, Jon A. Kemppainen³, Marion Kirk², and Elizabeth Wilson³

¹Dept. of Biology, Samford University, Birmingham, AL 35229

²Dept. of Biology, Univ. of Alabama at Birmingham, Birmingham, AL 35229

³Laboratories for Reproductive Biology and the Dept. of Pediatrics, Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC 27599

rljenkin@samford.edu, Phone (205) 726-2947, FAX (205) 726-2479

Effluent from a paper mill discharging into the Fenholloway River, Taylor County, Florida, USA, contains chemicals that masculinized females of the resident population of eastern mosquitofish, *Gambusia holbrooki*, as evidenced in females by elongated anal fins, normally a male-specific trait. To identify androgenic components in the effluent, free-flowing water was collected from the Fenholloway River and a control tributary in acid-washed carboys. Water samples were filtered through acid-washed glass wool and fractionated using C-18, solid phase extraction (SPE) and high performance liquid chromatography (HPLC: 20%-100% acetonitrile gradient in 0.25% H₃PO₄). The 80% and 90% methanol SPE fractions induced human androgen-receptor-dependent transcriptional activity in transient transfection cell culture assays. From these SPE fractions, two fractions collected from HPLC gradients induced androgen receptor transcriptional activity. Of these androstenedione was confirmed by liquid chromatography mass spectrometry (LCMS) with multiple reaction monitoring and was quantified by HPLC at concentrations of 0.14 nmoles/liter of effluent.