Report for 2005NE89B: Androgenic Growth Promoters in Nebraska Rivers: Detection and Toxicity

Publications

- Articles in Refereed Scientific Journals:
  - Kolok, AS, DD Snow, MK Sellin, S Kohno, LJ Guillette, Jr. " Steroidogenic compounds in the Elkhorn River Nebraska: Occurrence and impact." Manuscript to be submitted to Science of the Total Environment, Fall 2006.
- Conference Proceedings:
  - Kolok, A.S., D.D. Snow, S.Kohno, and L.J. Guillette, Jr. 2006. Clear as mud: Are cattle feedlots responsible for the defeminization of female fathead minnows in the Elkhorn River? VII Annual International Fish Congress on the Biology of Fishes. St. John's, Newfoundland, Canada. July 18-22.

Report Follows

Title: Growth promoters in Nebraska Rivers: Detection and Toxicity.

Project Number: 2005NE89B

Start Date: March 1, 2005

End Date: February 28, 2006

Funding Source: 104B

Research Category: Water Quality

Focus Categories: **Focus Categories**: AG, NPP, TS, WW, WQL

Descriptors: growth promoters, endocrine disruptors, fathead minnows, gene expression, passive integrative samplers

Primary PI: Alan S. Kolok

Other PIs: Daniel D. Snow

Project Class: Research

RESEARCH: In the last few years, there have been many reports throughout the United States suggesting that fish are being sexually altered by exposure to contaminants in the water. Male fish are being demasculinized, while females are being defeminized, most likely through the process of endocrine disruption. In rural areas such as Nebraska, the source of these biologically active compounds can include: effluent from wastewater treatment facilities, runoff from row crop agricultural landscapes, as well as runoff associated with animal feedlot operations. Understanding the environmental impact of contaminants from these sources requires both in-situ identification of the chemicals present and documentation of any effects on endocrine function to organisms. This study used novel in situ monitoring tools to detect and quantify steroid hormones and endocrine disruptive activity at several locations on a rural stream in northeast Nebraska.

During the summer of 2005, fathead minnows and polar organic chemical integrative samplers (POCIS) were deployed at four different locations during two time intervals (May and June). Two of the locations were adjacent to large cattle feedlots, a third location was immediately downstream from the Norfolk sewage treatment plant (STP) discharge structure, while the fourth site was located adjacent to a state wildlife management area (reference site). The POCIS, designed to trap and measure hydrophilic contaminants, and were deployed in triplicate in stainless steel canisters. Triplicate cages of fathead minnows were also deployed at each location, with 10 fish in each cage. During the May deployment, one POCIS canister and fathead minnows were lost at the Norfolk STP due to vandalism. In June, the POCIS and minnows deployed at the reference site was lost to a flood event. The remaining fish and POCIS were processed, analyzed and evaluated with respect to hormone levels and endocrine discruption activitiy.

Liquid chromatography-mass spectrometry was used to analyze extracts of the POCIS devices. Nanogram quantities of the natural estrogens 17β-estrodiol, estriol and estrone were detected in the POCIS at most sites, as was progesterone, testosterone, and 4-androstenedione. Low levels of the synthetic progesterone melangesterol acetate were detected in several of the samples, while the other synthetic steroids (17α-

ethynylestradiol, α-zearalonol, and 17β-trenbolone) were not. No consistent differences were apparent in the suite of compounds detected between locations in close proximity to feedlots and those of the control or the waste water treatment plant.

Fathead minnows deployed at each location were returned to the laboratory, their livers were dissected from the fish and differential gene expression was determined for a number of androgen- and estrogen-responsive genes. Quantification of gene expression was performed on: androgen receptor, estrogen receptor α, vitellogenin, cytochrome P450 3A, cytochrome P450 side chain cleavage, and steroidogenic acute regulatory protein. Vitellogenin and estrogen receptor α expression was depressed at all field sites relative to fish held in the laboratory, however this result may be due to attenuated development as much as due to exposure to steroidogenic compounds. In most cases, StAR, cytochrome P450 scc, and cytochrome P450 3A expression were significantly elevated in the fish caged in the vicinity of the feedlots and the wastewater treatment plant relative to fish held in the laboratory or fish caged at the reference site. As was true for the POCIS data, no consistent differences were apparent in gene expression between locations.

Steroidogenic compounds are found in the Elkhorn River system, and they are likely to be anthropogenic in origin. Proximity to feedlots or a wastewater treatment plant did not, however, lead to a specific "signature" in occurrence of steroidogenic compounds, nor in the effect that these compounds had on gene expression. The Elkhorn River appears to be systemically contaminated with steroidogenic compounds.