

EXhibit 8

**DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION 5**

**FY09 ENVIRONMENTAL CONTAMINANTS PROGRAM
ON-REFUGE INVESTIGATIONS SUB-ACTIVITY**

**Assessment of Endocrine Disruption in Smallmouth Bass (*Micropterus dolomieu*)
and Largemouth Bass (*M. salmoides*) in Region 5 National Wildlife Refuges**

Project ID: 5N44 (Year 2)

5n44 nationwide R5 endocrine on refuge interim fyo9.doc

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II. INTRODUCTION

The Problem

Since the early 1980s, endocrine disruption in humans, fish, and wildlife has been recognized as a global environmental concern (McLachlan 1980; Colburn and Clement 1992). Both man-made chemicals and plant and animal hormones have been shown to have endocrine disrupting effects. In aquatic systems, two major sources are agricultural production, especially intensive activities, such as animal feeding operations (AFOs) (Iwanowicz *et al.* 2004; Orlando *et al.* 2004) and sewage treatment plant effluents (e.g., Harries *et al.* 1996, 1997; Folmar *et al.* 1996, 2001). Kirk *et al.* (2002) found that sewage treatment plants with secondary treatment transfer up to 30 percent of the androgenic and estrogenic compounds into the effluent. In plants that relied solely on primary treatment, up to 93 percent of the androgenic and estrogenic compounds analyzed in influent were detected in effluent.

Evidence is mounting to suggest that endocrine disrupting compounds may be affecting reproduction in fish populations. Endocrine-disrupting effects in fish have been characterized through the use of biomarkers and by direct measurement of reproductive success. One key biomarker is the measurement of vitellogenin, a protein precursor of egg yolk (Harries *et al.* 1996). In male fish, vitellogenin concentrations are typically extremely low (non-detected or just above the limit of detection), whereas in male fish exposed to estrogenic compounds Vtg concentrations can be similar to those in females.

Studies in the Chesapeake Bay watershed

Studies of endocrine disruption in smallmouth and largemouth bass in the Potomac River watershed have received considerable attention in the media and resulted in Congressional hearings in October 2006 (USGS 2006). According to USGS (2006), the National Fish Health Research Laboratory (NFHRL) initiated fish health assessments of the South Branch of the Potomac River in 2003 and 2004 in response to fish kills and observations of external lesions on smallmouth bass and other species. The studies were a joint effort with the West Virginia Department of Natural Resources. In those studies, 16 of 24 sampling events showed more than 25% of the male bass with intersex characteristics.

In 2005, more extensive sampling to determine the extent of the intersex problem in the Potomac watershed was conducted. A portion of the efforts was funded through an Off-Refuge Investigation (Guy and Pinkney 2007). The 2005 sampling involved collaboration between USFWS-CBFO, USGS-NFHRL, Virginia Department of Game and Inland Fisheries, Virginia Department of Environmental Quality, West Virginia Department of Natural Resources, Maryland Department of Natural Resources, and Virginia Institute of Marine Sciences.

The Off-Refuge Investigation studied the effects of exposure to smallmouth and largemouth bass at five locations: Upper Conococheague Creek (agricultural), Lower

Conococheague Creek (sewage), Upper Monocacy River (agricultural), Lower Monocacy River (sewage), and the main stem of the Potomac River near the Blue Plains wastewater treatment plant in Washington, DC.

The most dramatic finding from 2005 was that 80-100 percent of male smallmouth bass and 30 percent of the male largemouth bass had intersex gonads (Table 1). Specifically, immature eggs were detected in the testes. In addition, fish from all sites (at similar reproductive stage) had relatively low gonadosomatic index (GSI) scores (GSI is the ratio between gonad weight and the total weight of the fish). These results suggest that bass populations in the Potomac River watershed may be experiencing decreased reproductive function. Chemistry data are expected shortly. In 2006, caged studies were performed with smallmouth bass in the Conococheague and Monocacy locations. Results are pending.

Endocrine Disruption and National Wildlife Refuges

Our goal is to evaluate the extent and magnitude of endocrine disruption (including intersex) in smallmouth and largemouth bass in rivers and impoundments at Region 5 National Wildlife Refuges.

The specific objectives of this project are to:

Years 1 and 2

Identify if fish, from selected locations in rivers on or adjacent to Region 5 NWRs are experiencing endocrine disruption as evidenced by gonad histopathology including intersex; abnormal hormone concentrations; or abnormal plasma vitellogenin concentrations.

To the extent possible, compare the findings in an upstream-downstream design to evaluate the impacts of suspected sources of endocrine-disrupting compounds.

Document whether fish are being exposed to endocrine-disrupting chemicals by performing *in vitro* screening assays on water samples at the collection sites.

Year 3

Identify if fish, from selected locations in ponds within Region 5 NWRs are experiencing endocrine disruption as evidenced by gonad histopathology including intersex; abnormal hormone concentrations; or abnormal plasma vitellogenin concentrations

Document whether fish are being exposed to endocrine-disrupting chemicals by performing *in vitro* screening assays on water samples at the collection sites.

Year 4

Integrate the results of Years 1 through 3 in a report. Prepare manuscripts and submit to journals. Prepare fact sheets and briefing statements for Congress. Work with public affairs to disseminate the results to print and electronic media. Local field offices work with refuges on specific problems and present findings to state agencies with designated Clean Water Act authority.

III. RESULTS TO DATE

Sampling for Year 1 has been coordinated with all participating field offices and their cooperators. A schedule has been prepared with sampling to begin the first week in September and end the last week in October. An Interagency Agreement is being prepared to provide funding to the National Fish Health Research Laboratory (NFHRL). Additional arrangements are being made to have a training session in mid-July at NFHRL and to coordinate sampling activities with the Lamar Fish Health Laboratory.

IV. SIGNIFICANT CHANGES TO ORIGINAL PROPOSAL

There are no significant changes.