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# AQUACULTURE DRUGS: 17 $\alpha$ - METHYLTESTOSTERONE TARGET ANIMAL SAFETY STUDY<sup>1</sup>

Project *Progress Report* for the Period  
December 15, 2004 to August 31, 2007

**NCRAC FUNDING:** \$50,000 (December 15, 2004 to December 31, 2007)

**PARTICIPANT:**

Anita M. Kelly	Southern Illinois University-Carbondale	Illinois
<i>Industry Advisory Council Liaison:</i>		
Rosalie A. Schnick	National Aquaculture NADA Coordinator	Wisconsin
<i>Extension Liaison:</i>		
Joseph E. Morris	Iowa State University	Iowa

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**PROJECT OBJECTIVES**

- (1) Interact with the Center for Veterinary Medicine (CVM) to determine the study design and protocol.
- (2) Submit the study protocol to CVM and gain acceptance from CVM for the study protocol.
- (3) Conduct a target animal safety study using 17 $\alpha$ -methyltestosterone (MT) on tilapia according to CVM guidelines for a target animal safety study in feed under good laboratory practices (GLP).
- (4) Write the final study report and submit to CVM through the MT Investigational New Animal Drug (INAD) Coordinator at Auburn University.
- (5) Provide progress reports to the North Central Regional Aquaculture Center (NCRAC).
- (6) Gain acceptance from CVM for the target animal safety study on MT in tilapia.

**ANTICIPATED BENEFITS**

The ability of aquaculturists to produce a fish that is uniform in growth and expends little energy toward reproduction will increase the profits and production from a facility. Currently, determination of the gender of tilapia by visual inspection is relatively difficult until the fish have obtained sexual maturity. It is well known that male tilapia grow faster than female

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<sup>1</sup>NCRAC has funded seven Aquaculture Drugs projects. A termination report for the first project is contained in the 1997-98 Annual Progress Report; a termination report for the second project is contained in the 1996-97 Annual Progress Report, a termination report for the third project is contained in the 2001-02 Annual Progress Report, and a termination report for the fourth project as well as a progress report for the seventh project are contained elsewhere in this report. A fifth project, which provided \$60,000 for a portion of the funds required to purchase sufficient radiolabeled AQUIS<sup>®</sup> for use in a total residue depletion study in rainbow trout, is also reported on under the progress report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) elsewhere in this report. This progress report is for the sixth Aquaculture Drugs project which is being undertaken by Anita M. Kelly. It is a 2-year project that began December 15, 2004.

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tilapia. Feeding tilapia a diet containing 17 $\alpha$ -methyltestosterone (MT) at the onset of exogenous feeding and prior to sexual differentiation has been shown to be a viable method of producing all male populations. Currently, the U.S. Fish and Wildlife Service (USFWS) Aquatic Animal Drug Approval Partnership Program (AADAP) holds the INAD for the use of MT in sex inversion in tilapia. As part of the drug approval process, a target animal safety study must be conducted and approved by CVM. This study will complete the target animal safety study.

### **PROGRESS AND PRINCIPAL ACCOMPLISHMENTS**

#### ***OBJECTIVE 1***

The Principal Investigator for this project was in constant communication with CVM and the USFWS AADAP (which holds the INAD under which this research is being conducted) to design and develop an acceptable protocol.

#### ***OBJECTIVE 2***

The first protocol was submitted on August 8, 2005 to the USFWS Aquatic Animal AADAP which holds the INAD and must submit all protocols to CVM. They submitted the protocol on August 30, 2005. On December 7, 2005 CVM responded to the protocol submission, to AADAP, and found the protocol unacceptable. The AADAP forwarded the comments to Kelly on January 12, 2006. This correspondence included CVM's detailed explanation with a list of items they wanted corrected. CVM's concerns to the protocol were addressed and the protocol rewritten and sent to AADAP for review and comment. A revised protocol was sent to AADAP on May 2, 2006 and to CVM on May 16, 2006. The revised protocol was reviewed by CVM and the reply sent to AADAP on August 14, 2006. CVM found this protocol unacceptable and

the AADAP forwarded the concerns of CVM to Kelly on August 18, 2006. The protocol was revised to address the new concerns of CVM. This revised protocol was accepted by CVM in February 2007.

#### ***OBJECTIVES 3-6***

The target animal safety study was completed through the necropsy of the fish. Pathology has not been conducted on the fish used in this study. During the study, the laboratory was audited by the Food and Drug Administration (FDA) for GLP compliance. During the audit, the inspectors felt that too many fish were "missing" from the tanks. Cannibalism had been noted when apparent. The fish were netted from the tanks weekly, counted by two individuals, and the lengths of 10 random individuals were measured and recorded. In addition to the missing fish, the FDA audit noted several other noncompliant items including feed discrepancies and lack of an official assignment of a Study Director in the absence of the assigned Study Director.

During the course of the study, the Study Director was in communication with CVM regarding the problems with GLP inspection. It was decided on August 17, 2007 that CVM could not accept the study as conducted due to numerous noncompliant items.

#### **WORK PLANNED**

Kelly is no longer at Southern Illinois University-Carbondale (SIUC) and the National Coordinator for Aquaculture New Animal Drug Applications will work with CVM, SIUC, and Kelly in regard to finalizing an acceptable target animal safety study on MT in tilapia.

#### **IMPACTS**

The ability of aquaculturists to produce a fish that is uniform in growth and expends

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little energy toward reproduction will increase the profits and production from a facility. Currently, determination of the gender of tilapia by visual inspection is relatively difficult until the fish have attained sexual maturity. Sex reversal of fish prior to sexual differentiation would enable the production of monosex populations. Under an existing INAD, tilapia are being sex reversed using MT. However, in order for this hormone to be approved by the FDA, a target animal safety study must be conducted and approved by CVM.

**SUPPORT**

NCRAC provided \$50,000 to SIUC which was the entire amount of funding allocated for this project.

**PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTE**

See the Appendix for a cumulative output for all NCRAC-funded Aquaculture Drugs activities.

# **APPENDIX**

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## AQUACULTURE DRUGS

### *Publications in Print*

- Barry, T.P., A. Marwah, and P. Marwah. 2007. Stability of 17 $\alpha$ -methyltestosterone in fish feed. *Aquaculture* 271:523-529.
- Bernardy, J.A., C. Vue, M.P. Gaikowski, G.R. Stehly, W.H. Gingerich, and A. Moore. 2003. Residue depletion of oxytetracycline from fillet tissues of northern pike and walleye. *Aquaculture* 221:657-665.
- Malison, J.A., J.A. Held, L.S. Procarione, and M.A.R. Garcia-Abiado. 1998. The production of monosex female populations of walleye from intersex broodstock. *Progressive Fish Culturist* 60(1):20-24.
- Marwah, A., P. Marwah, and H. Lardy. 2005. Development and validation of a high performance liquid chromatography assay for 17 $\alpha$ -methyltestosterone in fish feed. *Journal of Chromatography B*:824:107-115.

### *Reports*

- Bernardy, J.A., C. Vue, and M.P. Gaikowski. 2000. Oxytetracycline residue depletion from walleye fillet tissue (CAP-98-00084-07). Submitted to the Center for Veterinary Medicine, U.S. Food and Drug Administration. 1,517 pp.
- Gaikowski, M.P., J.J. Rach, A. Moore, J. Hamilton, D. Smith, and T. Harder. 2002. Efficacy of hydrogen peroxide to control mortality associated with saprolegniasis on eggs of channel catfish (*Ictalurus punctatus*), paddlefish (*Polydon spahula*), smallmouth bass (*Micropterus dolomieu*), and walleye (*Stizostedion vitreum*). Study report submitted to the Center for Veterinary Medicine, U.S. Food and Drug Administration for supporting clinical field trials under INAD 10-023. 23 pp.
- Green, B.W. 1996. Direct review submission to Division of Toxicology and Environmental Science, Center for Veterinary Medicine, U.S. Food and Drug Administration in support of the Tilapia 17  $\alpha$ -Methyltestosterone INAD (INAD #9647 A0000, January 24, 1996).
- Kohler, C.C., A.M. Kelly, M.J. DeJesus, E.M. Carnevale, S.R. Syska, and W.M. Muhlach. 1998. The safety of 17  $\alpha$ -Methyltestosterone for induction of sex reversal in walleye. Final Report

of the Safety Study for INAD 9647 E0009 and E0011. 602 pp.

- Rach, J.J., M.P. Gaikowski, and V.K. Dawson. 2002. Freedom of Information summary: Perox-Aid for the treatment of external flavobacter infections on all freshwater finfish. Submitted to the Center for Veterinary Medicine, U.S. Food and Drug Administration for INAD 10-023.

### *Manuscripts*

- Barry, T.P., A. Marwah, and P. Marwah. Fate of 17 $\alpha$ -methyltestosterone in water sediment systems under aerobic and anaerobic conditions. *Environmental Science and Technology*.
- Marwah, A., P. Marwah, H. Lardy, and T.P. Barry. Development and validation of a LC-MS assay for measuring very low concentrations of 17 $\alpha$ -methyltestosterone in water. *Journal of Chromatography*.

### *Papers Presented*

- Barry, T.P., A. Marwah, and P. Marwah. 2006. 17 $\alpha$ -methyltestosterone: product chemistry. 12<sup>th</sup> Annual Drug Approval Coordination Workshop, and National Aquaculture Drug Research Forum, La Crosse, Wisconsin, August 1-2, 2006.
- Barry, T.P., A. Marwah, and P. Marwah. 2006. 17 $\alpha$ -methyltestosterone: environmental safety. 12<sup>th</sup> Annual Drug Approval Coordination Workshop, and National Aquaculture Drug Research Forum, La Crosse, Wisconsin, August 1-2, 2006.
- Barry, T.P., A. Marwah, and P. Marwah. 2007. Measurement and stability of 17 $\alpha$ -methyltestosterone in fish feed. *Aquaculture* 2007, San Antonio, Texas, February 26-March 2, 2007.
- Barry, T.P., A. Marwah, and P. Marwah. 2007. Fate of 17 $\alpha$ -methyltestosterone in water/sediment systems. *Aquaculture* 2007, San Antonio, Texas, February 26-March 2, 2007.
- Barry, T.P., P. Marwah, and A. Marwah. 2007. 17 $\alpha$ -methyltestosterone: product chemistry. 13<sup>th</sup> Annual Drug Approval Coordination Workshop, and National Aquaculture Drug Research Forum, Bozeman, Montana, July 31-August 1, 2007.
- Barry, T.P., P. Marwah, and A. Marwah. 2007. Fate of 17 $\alpha$ -methyltestosterone in water/sediment systems. 13<sup>th</sup> Annual Drug Approval

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- Coordination Workshop, and National Aquaculture Drug Research Forum, Bozeman, Montana, July 31-August 1, 2007.
- Bernardy, J.A., C. Vue, J.R. Meinertz, M.P. Gaikowski, G.R. Stehly, S.L. Greseth, N.J. Spanjers, and W.H. Gingerich. 2000. Residue depletion of oxytetracycline from fillet tissues of coho salmon, walleye, and northern pike. 41<sup>st</sup> Annual Western Fish Disease Workshop, Gig Harbor, Washington, June 28-29, 2000.
- Gaikowski, M.P., M. Drobish, J. Hamilton, T. Harder, L.A. Lee, C. Moen, A. Moore, D. Smith, and J.J. Rach. 2001. Evaluation of the efficacy of hydrogen peroxide to control mortality associated with saprolegniasis on eggs of cool- and warmwater fish. Mid-Continent Warmwater Fish Culture Conference, Council Bluffs, Iowa, February 2001.
- Kelly, A.M. 2006. Progress on the Target Animal Safety Study for 17 $\alpha$ -methyltestosterone. Aquaculture America 2006, February 13-16, 2006, Las Vegas, Nevada.
- Kohler, C.C., A.M. Kelly, E.M. Carnivale, and W.L. Muhlach. 1997. Target animal safety studies for aquaculture. 28<sup>th</sup> Annual Meeting of the World Aquaculture Society, Seattle, Washington, February 19-23, 1997.
- Malison, J.A. 1997. Reproduction and sex reversal in yellow perch and walleye. 1997 North Central Aquaculture Conference, Indianapolis, Indiana, February 6-7, 1997.
- Marwah, A., P. Marwah, and H. Lardy. 2005. Validated LC-MS methods for the quantitation of 17 $\alpha$ -methyltestosterone in fish feed: application of multifactorial experimental design. American Society of Mass Spectroscopy, San Antonio, Texas, June 5-9, 2005 (poster presentation).
- Rach, J.J. 2001. Application of hydrogen peroxide treatment regimens. U.S. Fish and Wildlife Service Region Three Fisheries Biologists meeting, La Crosse, Wisconsin, September 5, 2001.
- Rach, J.J., and M.P. Gaikowski. 2001. An overview of hydrogen peroxide research and techniques used to ensure accurate application of chemical treatment regimens. Minnesota Aquaculture Association, Minneapolis, Minnesota, February 23-24, 2001.
- Rach, J.J., M.P. Gaikowski, and C.A. Perkins. 2001. Hydrogen peroxide, a potential broad spectrum therapeutant for treatment of fish diseases. Aquaculture America '01, Orlando, Florida, January 21-25, 2001.
- Riche, M., and D.L. Garling, Jr. 1999. Digestibility and retention of nitrogen in tilapia (*Oreochromis niloticus*) fed phytase treated soybean meal in a recirculating system. 30<sup>th</sup> Annual Meeting of the World Aquaculture Society, Sydney, Australia, April 26-May 2, 1999.