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# AQUACULTURE DRUGS: 17 $\alpha$ - METHYLTESTOSTERONE FEED STABILITY AND WATER BIODEGRADATION STUDIES<sup>1</sup>

Project *Termination Report* for the Period  
June 1, 2004 to August 31, 2007

**NCRAC FUNDING:** \$223,677 (June 1, 2004 to June 30, 2006)

**PARTICIPANTS:**

Terence P. Barry	University of Wisconsin-Madison	Wisconsin
Ashok Marwah	University of Wisconsin-Madison	Wisconsin
Padma Marwah	University of Wisconsin-Madison	Wisconsin

***Industry Advisory Council Liaison:***

Mark Willows	North American Fish Farmers Coop., Binford	North Dakota
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***Extension Liaison:***

Laura G. Tiu	Ohio State University	Ohio
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**REASON FOR TERMINATION**

The project objectives were completed.

(3) Gain acceptance from the Center for Veterinary Medicine (CVM) for the series of stability studies.

**PROJECT OBJECTIVES**

(1) Develop a robust and validated high performance liquid chromatography (HPLC) and liquid chromatography-mass spectroscopy (LC-MS) method to measure 17 $\alpha$ -methyltestosterone (MT) in fish feed.

(4) Review and develop an LC-MS method for detecting MT in water.

(5) Conduct a biodegradation study of MT in water.

(2) Conduct a series of stability studies on MT in fish feed (note: after receiving NCRAC funding to conduct the stability study, it was learned that two additional feed studies must also be completed: (1) a feed homogeneity study and (2) a feed segregation study).

(6) Gain acceptance from CVM for the biodegradation study on MT.

**PRINCIPAL ACCOMPLISHMENTS**  
***OBJECTIVE 1***

A method to measure MT in fish feed has been developed. The method has already

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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 and a termination report for the third project is contained in the 2001-02 Annual Progress Report. This termination report is for the fourth Aquaculture Drugs project which was chaired by Terence P. Barry. It was an 18-month project that began June 1, 2004. 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 reported on under the progress report for the National Coordinator for Aquaculture New Animal Drug Applications (NADAs) elsewhere in this report as are progress reports for the sixth and seventh projects.

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been published in a peer-reviewed journal. The method was accepted by the CVM for use in the studies proposed under Objective 2. The method has been transferred to CanTest, Ltd (a Canadian analytical company that has worked with MT in the past) for use in efficacy studies.

### ***OBJECTIVE 2***

A detailed experimental protocol was written describing the required studies (i.e., the feed stability, homogeneity, segregation experiments) and submitted to and accepted by CVM. All feed studies were completed according to the protocol and samples have been analyzed. The results indicated that (1) MT-treated feed prepared by the manufacturer (Rangen, Inc.) is homogeneously mixed during the manufacturing and bagging process, (2) MT remains uniformly distributed throughout the feed during prolonged storage at room temperature, and (3) MT concentrations in fish feed are stable for at least several months when feed is stored at 4.0°C (39.2°F) or lower, but decline linearly with time at higher temperatures. The half-life of MT (i.e., the time required for the MT concentration to fall from 60 mg/kg [ppm] to 30 mg/kg [ppm]) was 1.1 and 4.8 months for feed stored at 40.0°C (104.0°F) and 22.0°C (71.6°F), respectively. A scientific paper on the experimental results has been published.

### ***OBJECTIVE 3***

The MT Feed Study Report will be submitted to CVM in November 2007.

### ***OBJECTIVE 4***

The LC-MS method to detect MT in water/sediment has been completed and validated. The method will be submitted to the CVM along with the results of the water/sediment experiments.

### ***OBJECTIVE 5***

The investigators received final CVM approval for the water/sediment study protocol in November 2005. All of the water/sediment experiments have been conducted. For each experiment, both water and the sediments must be analyzed separately. To date, all of the water samples from both the aerobic and anaerobic experiments have been analyzed.

A summary of the biodegradation studies is as follows. Two sediment samples that differed with respect to total organic carbon and texture (“sand” and “clay”) were exposed to radioinert MT or [<sup>14</sup>C]-radiolabeled MT (<sup>14</sup>C-MT), under both aerobic and anaerobic conditions, for up to 56 days, to characterize the fate of MT in the aquatic environment. Samples were extracted and MT concentrations were quantified by a highly sensitive liquid LC-MS method or by radio-high performance radio chromatography using an on-line flow radiation analyzer. Total radiation was quantified by liquid scintillation counting. The data suggest that MT entering the aquatic environment is converted into metabolites that become tightly associated with the sediment. Half-lives for MT dissipation in the sediment systems ranged from 2–9 days, depending on the sediment type and the presence of oxygen. Sediment type had little effect on MT dissipation. The mineralization of MT under aerobic conditions was low (<9% conversion of MT to CO<sub>2</sub>). A scientific paper on the “MT Water Study” is in preparation.

### ***OBJECTIVE 6***

The MT Water Method Development and MT Water Degradation Study Reports were submitted to the CVM in October 2007. All work was conducted according to University of Wisconsin-Madison protocols.

## **AQUACULTURE DRUGS**

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### **IMPACTS**

MT is used to manipulate the gender of a variety of fish species cultured in the U.S., including tilapia, hybrid striped bass, yellow perch, sunfish, and esocids. The U.S. tilapia industry relies on the production of all-male populations, which grow significantly faster than mixed-sex populations. In this regard, this work is a critical contribution to the effort to obtain an original New Animal Drug Application approval for MT in tilapia. Once MT is approved for used in tilapia, approval for other key aquaculture species will follow.

### **RECOMMENDED FOLLOW-UP ACTIVITIES**

None are required or anticipated at this time.

### **SUPPORT**

NCRAC funds provided \$223,677 to the University of Wisconsin-Madison which was the entire amount of funding allocated for this project.

### **PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED**

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.