AQUACULTURE DRUGS: DETERMINATIVE METHOD FOR THE AQUI-S® MARKER RESIDUE IN FILLET TISSUE[1]

Project ***Progress Report*** for the Period
January 1, 2006 to August 31, 2007

NCRAC FUNDING: $129,936 (January 1, 2006 to December 31, 2006)

PARTICIPANTS:

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PROJECT OBJECTIVES

(1) Interact with the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM) to determine the requirements and procedures to develop and validate a determinative analytical method for the AQUI-S® marker residue in all cool and warm water species of fin fish.

(2) Develop and validate a determinative analytical method for the AQUI-S® marker residue in all cool and warm water species of fin fish according to CVM guidelines for method development under Good Laboratory Practices.

(3) Write the final study report and submit the report to an Investigational New Animal Drug (INAD) number established by CVM for AQUI-S®.

(4) Gain acceptance from CVM for the determinative analytical method for the AQUI-S® marker residue that will help support the approval of AQUI-S® for short-exposure handling for all cool and warm water species of fin fish.

ANTICIPATED BENEFITS

Currently, Finquel (MS-222) is the only fish anesthetic approved by the U.S. Food and Drug Administration (FDA). Use of this anesthetic is constrained by a 21-day withdrawal period. A critical need for use of an anesthetic with a short withdrawal

[1]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 sixth 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 AQUI-S® 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 seventh Aquaculture Drugs project which is being undertaken by Jeffrey R. Meinertz. It is a 1-year project that began January 1, 2006.

time in U.S. public aquaculture and fishery management has been expressed. A shorter withdrawal anesthetic would allow anesthetized fish to be handled and released immediately after conducting nearly all aquaculture and fishery management procedures including transport, spawning, marking, harvesting, and grading. AQUI-S® is a fish anesthetic under investigation as a short withdrawal time anesthetic.

PROGESS AND PRINCIPAL ACCOMPLISHMENTS

A study protocol was developed and submitted to CVM. They returned protocol the with their review comments which were used to revise the protocol.

Nearly all supplies needed to conduct the study were procured. Fish-rearing practices were modified to maximize fish growth so fish would be of an adequate size for the study.

A study records system was developed for the storage of data generated during the study. The chemical purity of the test chemical was verified with high performance liquid chromatography techniques.

The instrument (high performance liquid chromatography system) detection and quantitation limits were determined for isoeugenol analytical standards prepared with 90:10 methanol:water.

The loss of isoeugenol from solutions prepared with 90:10 methanol:water was evaluated periodically through a 21-day storage period.

Fillet tissue from unexposed fish was acquired from the following species: brown trout, channel catfish, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch. The fillet tissue from each species was homogenized with dry ice in preparation for impending studies requiring homogenized control fillet tissue.

Homogenized control fillet tissue from lake trout was processed with the proposed determinative method for an evaluation of chromatographic interference that would interfere with the determination of isoeugenol concentrations in lake trout fillet tissue.

Brown trout, channel catfish, hybrid striped bass, lake trout largemouth bass, northern pike, walleye, and yellow perch were exposed to Aqui-S® (a separate exposure for each species) for the purpose of generating endogenous isoeugenol residues in the fillet tissue. Generation of fillet tissue with endogenous isoeugenol was necessary for the evaluating method precision with fillet tissue containing endogenous isoeugenol residues and for evaluating isoeugenol stability in fillet tissue stored at <-70°C (-94°F).

The precision of the proposed determinative method was evaluated with brown trout, channel catfish, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch fillet tissue containing endogenous isoeugenol.

The loss of isoeugenol from fillet tissue containing endogenous isoeugenol and stored for about 1 month at <-70°C (-94°F) was evaluated with brown trout, channel catfish, hybrid striped bass, and lake trout fillet tissue.

Fillet tissue from unexposed fall Chinook salmon was acquired and homogenized with dry ice in preparation for impending studies requiring homogenized control fillet tissue.

Samples of homogenized control fillet tissue from the following species were processed with the proposed determinative method for an evaluation of fillet constituents that would interfere with the determination of isoeugenol concentrations: brown trout, channel catfish, fall Chinook salmon, hybrid striped bass, largemouth bass, northern pike, walleye, and yellow perch.

The method detection and quantitation limits were determined with isoeugenol fortified fillet tissue as were method accuracy and within day precision from the following species: brown trout, channel catfish, fall Chinook salmon, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

The method accuracy and within day precision were determined with isoeugenol fortified fillet tissue from the following species: brown trout, channel catfish, Fall Chinook salmon, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

The method day-to-day precision was determined with isoeugenol fortified fillet tissue from channel catfish.

The loss of isoeugenol from fillet tissue extracts from the following species was determined after 1, 7, and 14 days: brown trout, channel catfish, fall Chinook salmon, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

The loss of endogenous isoeugenol from fillet tissue from the following species and stored at <-70°C (-94°F) was determined after 1, 2, 3, 4, 5, and 6 months: largemouth bass, northern pike, walleye, and yellow perch.

The loss of endogenous isoeugenol from fillet tissue from the following species and stored at <-70°C (-94°F) was determined after 2, 3, 4, 5, and 6 months: brown trout, channel catfish, hybrid striped bass, and lake trout.

The loss of endogenous isoeugenol from fillet tissue from the following species was determined after subjecting fillet tissue to three freeze/thaw cycles: brown trout, channel catfish, hybrid striped bass, lake trout, largemouth bass, northern pike, walleye, and yellow perch.

A comprehensive final report describing the study results has been assembled and reviewed for accuracy and compliance with FDA regulations for good laboratory practices by the UMESC Quality Assurance Officer.

WORK PLANNED

Because of the following statement issued in late April, 2007, submission of the report to CVM for review and submission to INAD number 11-475 for AQUI-S® has been postponed:

> "**STATEMENT ON ISOEUGENOL (AQUI-S®)**
>
> Isoeugenol (the active ingredient in AQUI-S®) has been under evaluation by the National Toxicology Program (NTP), an interagency program whose mission is to evaluate chemical agents for potential public health risks. Recently, NTP was forced to delay the review of their nearly completed two-year toxicology studies on isoeugenol until February 2008 because of higher priorities. Although the study data have not been fully analyzed, the preliminary assessments of the data do not eliminate the possibility that isoeugenol residues

in treated fish could pose a human health risk.

Because we need to be absolutely certain that there are no human food safety issues that would preclude the approval of AQUI-S®, the U.S. Fish & Wildlife Service (FWS) and the U.S. Geological Survey (USGS) and other participating partner groups have agreed to institute interim measures that will be effective until the NTP meeting in February 2008. Effective April 27, 2007, all ongoing and planned AQUI-S® research funded under the Association of Fish and Wildlife Agencies' Multi-State Conservation Grant, and allied work supported with federal base funds of FWS and USGS will be suspended until the completion of the NTP review. Additionally, FWS will temporarily suspend all field activities under their Investigational New Animal Drug exemption for AQUI-S® until the NTP review is complete. Although the decision to temporarily suspend all publicly funded AQUI-S® research activities was not an easy decision to make, as responsible stewards of public funds it is the correct course of action. It should be noted that significant portions of the data necessary to address many of the original AQUI-S® goals and objectives of the Federal-State Aquaculture Drug Approval Partnership Project have already been generated. It is also important to note that USGS is constrained from further development of residue chemistry data until a tolerance value for the residues has been established by the Center for Veterinary Medicine. This work cannot be initiated until the results of the NTP studies are finalized. We look forward to the opportunity of continuing our collaborative AQUI-S® research efforts in February 2008."

A decision to submit the final report will be made after the National Toxicological Program has made a ruling concerning the status of isoeugenol posing a human health risk.

IMPACTS

To support FDA approval of a new animal drug for fish, a series of toxicology and residue chemistry studies are conducted to demonstrate the safety of food products derived from treated fish. Mammalian toxicology studies basically determine if the drug is safe for humans to consume and the amount of drug residues that can be consumed daily for a lifetime without experiencing adverse effects (acceptable daily intake; ADI). Considering the amount of tissue consumed in a lifetime, the ADI is used to calculate a safe concentration for all of the drug's residues in the edible tissue. Residue chemistry studies are conducted to assess drug residues in the edible fillet tissue from treated fish. First a total drug residue depletion study is conducted resulting in a identification of all of the drug's residues in the edible fillet tissue and characterization of the depletion of those residues from the fillet. Based on data from this study a marker residue is selected. The marker residue is one compound or group of compounds that will represent all of the drug's residues in subsequent depletion studies.

After selection of a marker residue, analytical methods for the marker residue are developed and validated. Two methods are required, a determinative method (activities described in this report were conducted to fulfill requirements for a determinative method) and a confirmatory method. The determinative method determines concentrations of the marker residue in edible tissue. The confirmatory

method confirms the results from the determinative method and irrefutable identifies the marker residue in the tissue.

After validating a determinative method, marker residue depletion studies are conducted. Data from these studies are used in conjunction with the safe concentration to determine a tolerance concentration for the marker residue, as well as a withdrawal time. The tolerance concentration is the concentration of the marker residue in the edible tissue that represents the safe concentration (the concentration of all drug residues that is considered to be safe). The withdrawal time is the time it takes for the fish to deplete all drug residues to the safe concentration.

CURRENT STATUS
The total residue depletion study was completed in 2005. Based on the results from the total residue depletion study, isoeugenol will most likely be selected as the marker residue. However, because of the information described in the notification previously presented, all FDA decisions concerning AQUI-S® have been postponed. Nonetheless, all work validating a determinative method for the probable marker residue was completed. With the completion of that work, we are now poised to develop and validate a confirmatory method for the probable marker residue as well as conduct the marker residue depletion studies. Because of the decision to stop all work concerning AQUI-S®, we cannot continue developing data for AQUI-S®.

If a decision is made to continue work with AQUI-S®, the next steps toward an approval will be for FDA to calculate an ADI, calculate a safe concentration, accept data from the total residue depletion study, officially select isoeugenol as the marker residue, and review and accept data from the validation of the determinative method. Additionally, a confirmatory method will need to be developed and validated for the marker residue and conduct at least 3 marker residue depletion studies. Data from that work will be submitted to the FDA for their review and acceptance of that data should mark the end of work for an AQUI-S® approval.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED
See the Appendix for a cumulative output for all NCRAC-funded Aquaculture Drugs activities.

SUPPORT

YEAR	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2006	$129,936			$30,044[a]		$30,044	$159,980
TOTAL	$129,936			$30,044		$30,044	$159,980

[a]Estimate of additional UMESC salary costs for a GS13 and GS11 (4 pay periods each) that will be accrued during the 4th quarter of calender year 2006.

APPENDIX

AQUACULTURE DRUGS

Publications in Print

Barry, T.P., A. Marwah, and P. Marwah. 2007. Stability of 17α-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 17α-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 dolomieui*), 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 α-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 α-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 17α-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 17α-methyltestosterone in water. Journal of Chromatography.

Papers Presented

Barry, T.P., A. Marwah, and P. Marwah. 2006. 17α-methyltestosterone: product chemistry. 12th 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. 17α-methyltestosterone: environmental safety. 12th 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 17α-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 17α-methyltestosterone in water/sediment systems. Aquaculture 2007, San Antonio, Texas, February 26-March 2, 2007.

Barry, T.P., P. Marwah, and A. Marwah. 2007. 17α-methyltestosterone: product chemistry. 13th 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 17α-methyltestosterone in water/sediment systems. 13th Annual Drug Approval

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. 41st 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 17α-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. 28th 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 17α-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. 30th Annual Meeting of the World Aquaculture Society, Sydney, Australia, April 26-May 2, 1999.