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

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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Extension Liaison:
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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

1NCRAC 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.

PROGRESS 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 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,
isoegenol 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 isoegenol 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

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*aEstimate of additional UMESC salary costs for a GS13 and GS11 (4 pay periods each) that will be accrued during the 4th quarter of calendar year 2006.
APPENDIX
AQUACULTURE DRUGS

Publications in Print


Reports


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


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.


Papers Presented


