**Project Title**: Efficacy of Eugenol (AQUI-S®20E) to Reduce Transport Stress and Mortality of Tilapia and Yellow Perch [Termination Report]
**Key Word(s):** Aquaculture Drugs
**Total Funds Committed:** $100,000
**Initial Project Schedule:** September 1, 2011 to August 31, 2013
**Current Project Year:** September 1, 2015 to August 31, 2016
**Participants:** Mark P. Gaikowski, USGS Upper Midwest Environmental Sciences Center, Wisconsin; Christopher F. Hartleb, University of Wisconsin – Stevens Point, Wisconsin
**Industry Liaison:** Mark Willows, Binford Eagle Fisheries, North Dakota
**Reason for Termination:** Project objectives completed and funds have been terminated.

## Project Objectives

1. Interact with CVM to determine the study design and protocol needed to develop the effectiveness data to support a transport sedative claim for eugenol for selected finfish species. The protocol must comply with current CVM Guidance For Industry for the development of pivotal effectiveness data and the study data collection must with CVM Good Clinical Practices regulations.
2. Obtain fully disclosable Investigational New Animal Drug (INAD) exemptions for the selected sedative to be tested from CVM.
3. Obtain Categorical Exclusions from the requirement to complete an Environmental Assessment or complete an Environmental Assessment for the selected sedative prior to its use and receive concurrence from CVM Environmental Safety Team.
4. Submit the pivotal effectiveness protocol to CVM for concurrence.
5. Conduct pivotal effectiveness studies using the selected sedative on finfish species according to the CVM-concurred protocol and in compliance with CVM Good Clinical Practices regulations.
6. Summarize the study data into a Final Study Report (FSR) and archive all study data in publicly accessible archives
7. Submit the FSR to the publicly disclosable INAD file provided by CVM and request CVM review of the FSR and concur that the effectiveness technical section is complete for the selected sedative.
8. Respond to CVM comments on the FSR to ultimately obtain concurrence that the effectiveness technical section is complete for the use of the selected sedative as a transport sedative for the selected species.
9. Prepare a Freedom of Information summary of the submitted data and provide it to CVM.

## Project Summary

Fish transport costs are a substantial portion of the operational expenses in the aquaculture industry in the North Central Region (NCR). Increasing fish loading density during transport could substantially increase the efficiency of NCR aquaculture operations by enabling the transport of more fish per unit of fuel. This project was undertaken to determine if eugenol sedation would benefit fish transport procedures to improve fish welfare and post-transport survival on two economically important fish in the NCR.

**Technical Summary and Analysis**

This project was completed through a series of three studies: (Study #1) to determine the physiological effects of eugenol on yellow perch (*Perca flavescens*) and tilapia, (Study #2) to determine the anesthetic (behavioral) effects of eugenol on yellow perch and tilapia, and (Study #3) to determine the survival of yellow perch and tilapia transported under eugenol sedation. Results from Study #1 are published in Cupp et al. (2016a). This study evaluated the effects of AQUI- S®20E (10% eugenol) on the mass specific metabolic rates of yellow perch and Nile tilapia using static respirometry. In 17°C (62.6$^{O}$F) water and loading densities of 60, 120, and 240 g/L (0.5, 1.0, and 2 lb/gal), yellow perch control groups (0 mg/L eugenol) had metabolic rates of 329.6-400.0 mg O2/kg/h, while yellow perch exposed to 20 and 30 mg/L eugenol had significantly reduced metabolic rates of 258.4-325.6 and 189.1-271.0 mg O2/kg/h, respectively. Nile tilapia immersed in 30 mg/L eugenol had significantly reduced metabolic rates (424.5±42.3 mg O2/kg/h) relative to control fish (546.6±53.5 mg O2/kg/h) at a loading density of 120 g/L in 22°C water. Metabolic rates at 240 and 360 g/L loading densities were similar across all sedation levels for Nile tilapia. Results from this study demonstrated that eugenol reduced the metabolic rates of yellow perch at high loading densities, but Nile tilapia showed only minor suppression of metabolic rates in response to eugenol sedation. Results from

Study #2 is published in Cupp et al. (2016b). This range finding study evaluated combinations of loading densities, eugenol concentrations, and exposure durations to determine the anesthetic effects on yellow perch and Nile tilapia using static exposures. Yellow perch were immersed in 0, 10, 20, and 30 mg/L eugenol for 2, 6, and 10 h at 120, 240, and 360 g/L (1, 2, and 3 lb/gal) loading densities.

Nile tilapia were immersed in 0, 10, 20, and 30 mg/L eugenol for 2, 6, and 10 h at 240, 360, and 480 g/L (2, 3, and 4 lb/gal) loading densities. In general, eugenol depleted rapidly from static exposure tanks regardless of starting concentration, while sedation levels were highly varied. Yellow perch immersed in 20 and 30 mg/L eugenol were lightly sedated (i.e. reduced swimming and startle responses with equilibrium maintained) for up to 7h during static exposure. However, immersion in 30 mg/L also induced loss of equilibrium in yellow perch; an unwanted endpoint during live transport due to the risk of suffocation. Nile tilapia sedation was modest at these same concentrations and all fish were fully recovered within 2 h of static exposure.

Collectively, this study found that eugenol is effective to sedate high loading densities of yellow perch and Nile tilapia, but sedation levels vary with species, loading density, and starting eugenol concentration. Results from Study #3 are currently in journal review. Briefly, this study determined the effectiveness of eugenol sedation during live transport to enhance post-transport survival relative to unsedated fish. Fish were transported in 0, 10, and 20 mg/L eugenol for 6 h at 240 g/L (yellow perch) and 480 g/L (Nile tilapia) across various roads, highways, and interstates. Similar to Study #2, eugenol depleted rapidly from transport tanks and the behavioral effects of sedation were short-lived for both species. Survival was >98% for both species up to 14-d post-transport. No differences in survival between sedated fish and unsedated fish were found.

**Principal Accomplishments**

Objective 1. — Upper Midwest Environmental Science Center (UMESC) collaborated with Center for Veterinary Medicine (CVM) and developed an acceptable protocol and study design for generating non-pivotal effectiveness data. UMESC submitted the protocol to CVM through

the UMESC publicly-disclosable Investigational New Animal Drug (INAD) permits for AQUI-S®20E and requested an informal CVM review prior to conducting the study. CVM staff were uncertain about how to assess a potential label claim and data generated through non-pivotal effectiveness trials would be important for development of a pivotal effectiveness study. Data and reports from non-pivotal effectiveness trials and a draft pivotal effectiveness protocol were submitted to CVM in January of 2014.

Responses from CVM regarding these submissions were received May 2014. Briefly, CVM response indicated that non-pivotal data informed them on both effectiveness and target animal safety. Suggestions concerning future studies under these conditions were to use fewer response variables. Itemized comments for revisions of the pivotal effectiveness protocol were addressed to work toward protocol concurrence from CVM and a revised pivotal effectiveness protocol was submitted in February 4, 2015. On March 27, 2015, CVM did not concur with the revised protocol submission. Although CVM agreed with overall experimental design, protocol concurrence because no official label claim was put forth by AQUI-S New Zealand Ltd. (product sponsor) specifying the use of AQUI-S20E® for fish transport. No further protocol concurrence will be issued by CVM until a label claim is developed; regardless of their agreement with study design.

Objective 2. — All protocols, data, and final study reports submitted to CVM will be submitted by UMESC to INAD 011-766.

Objective 3. — Work within this objective is dependent on progress made by the drug sponsor on completion of an original Environmental Assessment for the use of AQUI-S® 20E.

Objective 4. — A revised pivotal effectiveness protocol was submitted based on input from CVM, UMESC and the drug sponsor (AQUI-S New Zealand Ltd.). The CVM non-concurrence letters were received. See description in Objective 1.

Objective 5. — Pivotal effectiveness study was completed in Summer 2015. However, protocol concurrence with study design was not attained prior to study initiation for reasons described in Objective 1.

Objective 6. — FSR has been developed and is currently under peer-review with a scientific journal. Data archiving will take place upon acceptance of FSR.

Objective 7. — MESC will submit the FSR to CVM for review. As previously described, the effectiveness technical section will not be completed from this work because no label claim has been made by the drug sponsor.

Objective 8. — See line item 7 above.

Objective 9. — See line item 7 above.

**Impacts**
Results from this project describe considerations with sedating yellow perch and tilapia for live transport.

**Recommended Follow-Up Activities**
Further collaboration between industry and drug sponsors will be important for describing the needs for eugenol sedation during live transport and will be necessary to develop a label claim for FDA. Although this project determined that yellow perch and tilapia can be effectively sedated using 10-30 mg/L eugenol, no differences in post-transport survival between fish that were sedated or not sedated during live transport. Future research should also consider transporting fish under different circumstances (e.g. extreme temperatures, long transport durations, low dissolved oxygen), as the benefits of sedation on fish welfare are likely to be realized under more adverse conditions.

**Publications, Manuscripts, Workshops, and Conferences**
See the Appendix for a cumulative output for all NCRAC-funded Animal Drugs activities.