ATTACHMENT A

TOTAL RESIDUE DEPLETION STUDY FOR AQUI-S®

LEAD INSTITUTION: AQUI-S New Zealand, Ltd.

FUNDING LEVEL: $60,000

BACKGROUND AND JUSTIFICATION

There is no legally approved anesthetic with a zero withdrawal time (i.e., time between last treatment and potential consumption or release) available for the many procedures that are used in aquaculture production or fishery management. The only anesthetic currently approved by the U.S. Food and Drug Administration, Center for Veterinary Medicine (CVM) for fish is tricaine methanesulfonate (i.e., MS-222) but it has a 21-day withdrawal time and that does not allow its use for most of the aquaculture or fishery management procedures requiring anesthesia. Some groups have the misconception that clove oil or eugenol are legal to use for these procedures but CVM has declared that these two compounds are unapproved drugs and, thus, are illegal to use.

The procedures requiring zero withdrawal time include: (1) artificial spawning with carcasses allowed as fish meal for feeds or with live fish released for sport, commercial, or subsistence fisheries, (2) humane rested harvest of cultured fish to improve product quality and extend shelf life, (3) sorting brood fish, (4) marking, tagging, and immediate release or harvest of both cultured and captured fish, (5) facilitating vaccine administration to market-sized fish, (6) grading fish by size for immediate harvest or release, and (7) transport of market-size fish for immediate harvest or release.

AQUI-S®, an efficacious broad-spectrum anesthetic that contains isoeugenol as the active ingredient, has the potential to fulfill the need for a zero withdrawal anesthetic in the culture and management of fish and shellfish. To gain CVM approval of any drug, researchers must prove that it is safe and effective for its intended purpose. The data technical sections required for a new animal drug application (NADA) approval include: (1) product chemistry, (2) environmental safety, (3) mammalian safety, (4) human food safety, (5) target animal safety, and (6) efficacy. A consortium of interested private and government agencies are providing funds to address the above technical section requirements of AQUI-S® for aquaculture and fishery management purposes (see Table 1 for status of Technical Sections). These organizations and agencies include AQUI-S New Zealand Ltd. (sponsor of AQUI-S®), U.S. Geological Survey's Upper Midwest Environmental Sciences Center (UMESC), U.S. Fish and Wildlife Service's Aquatic Animal Drug Approval Partnership Program (AADAPP), International Association of Fish and Wildlife Agencies (IAFWA) through Multi-State Conservation Grants, certain state Department of Natural Resources that contributed to the Federal-State Aquaculture Drug Approval Partnership Project, and Michigan State University through contributions to the National Aquaculture NADA Coordinator position, CVM, Regional Aquaculture Centers, aquaculture organizations and associations, U.S. Department of Agriculture, and aquaculture growers.

The Board of Directors of the North Central Regional Aquaculture Center (NCRAC), based on input from the Center's Industry Advisory Council, indicated at both the 2003 and 2004 Annual Program Planning Meetings that funds should be made available for activities that would lead to aquaculture drug approvals. The top priority drugs as identified by NCRAC were 17α-Methyltestosterone (MT) and AQUI-S®. As identified in NCRAC's white paper on Salmonids, approval of AQUI-S® as a zero withdrawal anesthetic for all freshwater-reared salmonids is of high priority. Having the availability of an anesthetic compound is important to the industry because its use delays rigor mortis and consequently post-harvest spoilage (i.e., fish muscle becomes soft and gapes, reducing the yield). Thus, AQUI-S® can increase yield and consistency, prolong shelf life, and provide a product with greater appeal to consumers which is important to the 12-state North Central Region because a third of all farm-gate sales for food fish, based on the 1998 Census of Aquaculture, is of rainbow trout.

PROPOSED ACTIVITY

In February 2004, NCRAC's Board of Directors authorized up to $60,000, a portion of the total costs, to purchase radiolabeled AQUI-S® for use in a total residue depletion study in rainbow trout (a surrogate for all salmonids) as part of the drug approval efforts on this anesthetic drug. Even though the sponsor, AQUI-S New Zealand, has already expended a great amount of money on other technical sections for this compound, they have agreed to provide $50,000 of the $110,000 needed for the purchase of the material required to conduct the total residue depletion study. Undertaking this study is a critical first step in developing data to support the expensive human food safety technical section required by CVM. This study will be undertaken in La Crosse, Wisconsin by UMESC who has the funds for salaries, facilities, etc. but not the radiolabeled material. UMESC is the only facility in the country at the current time with the expertise, personnel, facilities, designated funding, and backing of the states and federal government to undertake this study. Data from this study will allow CVM to determine whether residues of concern will be present in edible tissues of salmonids at zero withdrawal as outlined below.

Total residue depletion studies are conducted to allow CVM reviewers to evaluate the concentrations and rates of depletion of all drug residues in edible tissues, both parent drug and metabolites, resulting from use-pattern exposure to the drug. In terms of experimental design, the studies are relatively direct; groups of animals are exposed to the radiolabeled drug at concentrations designed to mimic anticipated maximal doses of exposure. The edible tissues of treated animals are sampled after drug exposure and the tissues analyzed to establish a pattern of residues remaining in the tissues over time. As the regulatory agency for AQUI-S®, CVM has an interest in mimicking normal use conditions that provide for the maximum total drug residues in the animals during an individual exposure. CVM requires that (1) the fish used must be at or near market size (about 550 grams) to avoid size-related differences in tissue concentration in edible tissues of exposed fish and (2) the exposure should be conducted in baths containing the test article of sufficient size that that the concentration of the drug in the exposure solution will not drop below 90% of the initial treatment concentration during the exposure.

A sufficient number of market-sized rainbow trout must be exposed to isoeugenol in anesthetic baths to ensure that an adequate number of fish are sampled at the completion of exposure (generally 6 fish per group) and at times during the withdrawal period. Edible tissues from exposed fish are analyzed for total residues to establish patterns of accumulation and depletion for the residues that may be present in the tissues. Because of the number of market-sized fish to be used (a minimum of 30 fish), the volume of the exposure water must be concomitantly large to accommodate the animals. Moreover, the volume of exposure water must also be sufficiently large that the concentration of the radioactive test article will remain above 90% of the initial target exposure concentration in all exposure baths. These requirements place a burden on execution of the studies in that a large quantity of radiolabeled material is required to complete the studies.

Studies with anesthetics in fish are complicated by the fact that pharmacological effects of the drug can modify its own uptake, distribution, and excretion because the drug acts to reduce breathing rates and rhythmicity, gill perfusion efficiency, and depress cardiac performance. These effects generally will act to reduce tissue accumulation in fish treated at anesthetizing concentrations where gill ventilation and perfusion efficiency is reduced. Consequently, the design of experiments must consider maximized anesthetic accumulation (e.g., rested harvest where lower anesthetic concentrations are used for a more prolonged period of time) as the most probable boundary exposure scenario leading to maximum tissue drug residues. UMESC researchers will determine the exposure concentration of isoeugenol that maximizes drug residues in tissues from pilot studies that are currently in progress.

In previous studies, UMESC researchers have developed methods to extract total isoeugenol residues from tissues of exposed rainbow trout with recoveries exceeding 90% and a high performance liquid chromatography method to separates at least three residues other than the parent isoeugenol. The methods previously developed by UMESC researchers will be used to analyze tissues of fish exposed during the total residue depletion studies.

ANTICIPATED BENEFITS

Completion of this study will provide a key piece of information that will allow CVM to: (1) determine if residues in tissues immediately after exposure are sufficiently low to allow a zero withdrawal and (2) declare a marker residue in salmonids that can be used to assess depletion rates of residues if a withdrawal period is required. This study is central to addressing requirements of the human food safety technical section that will allow CVM to determine whether AQUI-S® can be used safely as a zero withdrawal anesthetic for use on all salmonids. If these data from the total residue depletion study are accepted by CVM, additional residue chemistry studies can be initiated that will ultimately result in the final approval for AQUI-S® for use on salmonids. If approved, AQUI-S® would be the only anesthetic that would be legal to use at a zero withdrawal time in all salmonid aquaculture and management operations requiring anesthesia. The approval of AQUI-S® will benefit the anticipated users of AQUI-S® including producers, managers, and researchers associated with salmonid production, enhancement, restoration, and research. The ultimate beneficiary will be the consumers who will be able to eat a safe product.

BUDGET

This request is for $60,000, a portion of the total funds needed to purchase radiolabeled AQUI-S® for a total residue depletion study to be conducted by UMESC.

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0022
Expires 5/31/98

BUDGET

ORGANIZATION AND ADDRESS AQUI-S New Zealand Ltd. 15011 NE 108th Place Redmond, WA 98052 **PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S)** Dr. Thomas D. Goodrich, U.S. Agent				**USDA AWARD NO.** Duration Proposed Months: 12 **FUNDS REQUESTED by PROPOSER**	Duration Awarded Months: _____ **FUNDS APPROVED BY CSREES** (If Different)
A. Salaries and Wages	**CSREES FUNDED WORK MONTHS**				$
1. No. of Senior Personnel	Calendar	Academic	Summer		
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates					
b. ___ Other Professional					
c. ___ Graduate Students					
d. ___ Prebaccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop and Other					
Total Salaries and Wages . . . . . →				$0	
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits (A plus B) . . . . . →				$0	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for **each** item.)					
E. Materials and Supplies				$60,000	
F. Travel 1. Domestic (Including Canada)					
2. Foreign (List destination and amount for each trip.)					
G. Publication Costs/Page Charges					
H. Computer (ADPE) Costs					
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) See Budget Narrative.					
J. Total Direct Costs (C through I) . . . . . →				$60,000	
K. Indirect Costs If Applicable (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)					
L. Total Direct and Indirect Costs (J plus K) . . . . . →				$60,000	
M. Other . . . . . →					
N. Total Amount of This Request . . . . . →				$60,000	$
O. Cost Sharing (If Required Provide Details)					

NOTE: Signatures required only for Revised Budget — This is Revision No. →

NAME AND TITLE (Type or print)	**SIGNATURE**	**DATE**
Principal Investigator/Project Director		
Authorized Organizational Representative		

Form CSREES-55 (6/95)

BUDGET NARRATIVE

E. Materials and Supplies. These funds ($60,000) would be a portion of the total costs necessary to puchase radiolabeled AQUI-S® for a total residue depletion study to be conduced at La Crosse, Wisconsin by the U.S. Geological Survey's Upper Midwest Environmental Sciences Center (UMESC).

Table 1. Status of Technical Sections needed for an initial NADA for AQUI-S® as a zero withdrawal anesthetic for all salmonids.

Technical Section	Entity—Data—Action	Impediments or Cost—Action
NADA Package	Sponsor and National Aquaculture NADA Coordinator—NADA package—planned	None—sponsor committed to acceptance
Product Chemistry	Sponsor—product chemistry package—planned	None—sponsor committed to acceptance
	Sponsor—full biological activity—submitted Fall 2003	None—sponsor committed to acceptance
Environmental Safety (pond/flow-through, saltwater)	Sponsor—biodegradation studies (freshwater & saltwater)—submitted 11/24/03	None—sponsor committed to acceptance
	Sponsor—Ecotoxicity & physico-chemical studies & environmental assessment—planned or in progress	None—sponsor committed to acceptance
Human Food Safety—Mammalian Toxicology	Sponsor—National Toxicology Program studies—monitoring studies due for completion Summer 2004 & peer review by Spring 2005; Sponsor—mammalian safety summary—planned	None—sponsor committed to acceptance
Human Food Safety—Residue Chemistry (Atlantic salmon)	Sponsor—residue profile/Atlantic salmon—completed, to be submitted soon	None—sponsor committed to acceptance
Human Food Safety—Radiolabeled material (freshwater-reared salmonids)	Sponsor & NCRAC—radiolabeled material for total residue depletion study—Sponsor partial support ($50,000) & NCRAC Board voted to fund 2/7/04 at $60,000; need concurrence from USDA	None—if USDA concurs with NCRAC Board funding request
Human Food Safety—Residue Chemistry (freshwater-reared salmonids)	UMESC—analytical method for isoeugenol and its metabolites in rainbow trout tissue, total residue depletion study, determinative method for marker residue in salmonids, marker residue depletion study—planned or in progress	None—pending acceptance—base & outside funds
Human Food Safety—Residue Chemistry (freshwater-reared salmonids)	Sponsor & IAFWA—confirmatory method for marker residue in salmonids—no activity because unknown if needed	Funds—may be needed if confirmatory method is required
Target Animal Safety (Atlantic salmon)	Sponsor—Atlantic salmon—completed, to be submitted soon	None—sponsor committed to acceptance
Target Animal Safety (freshwater-reared salmonids)	AADAPP—rainbow trout for all freshwater-reared salmonids—protocol to CVM 1/12/04	None—pending acceptance
Efficacy (Atlantic salmon)	Sponsor—Atlantic salmon—completed, to be submitted soon	None—sponsor committed to acceptance
Efficacy (freshwater-reared salmonids)	AADAPP—4 efficacy studies/salmonids—accepted as supportive (1) steelhead trout, (2) lake trout, mountain whitefish, & rainbow trout, (3) cutthroat trout, & (4) bull trout 1/29/04	None—pending acceptance
	AADAPP—2 pivotal efficacy studies/salmonids—submitted 10/2/03 (Chinook salmon), 1/20/04 (rainbow trout—handeable & anesthetized)	None—pending acceptance—base & outside funds
Freedom of Information (FOI) summary (Atlantic salmon)	Sponsor—FOI/anesthetic/Atlantic salmon—planned	None—sponsor committed to acceptance
FOI summary (freshwater-reared salmonids)	AADAPP—FOI/anesthetic/freshwater-reared salmonids—in progress	None—pending acceptance