Seventh Annual Report of Progress

Performance Period: July 1, 2000 to June 30, 2001

Approval of Drugs for Public Fish Production

a project of the

International Association of Fish and Wildlife Agencies (IAFWA)

by

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Executive Summary

The International Association of Fish and Wildlife Agencies Project (IAFWA Project) entitled "Federal-State Aquaculture Drug Approval Partnership Project" has now completed seven years and is in its eighth and final year. New Animal Drug Application (NADA) submissions to the Center for Veterinary Medicine (CVM) to support original or supplemental approvals for IAFWA Project drugs are the result of efforts by the Upper Midwest Environmental Sciences Center (UMESC), Harry K. Dupree Stuttgart National Aquaculture Research Center (HKD-SNARC), U.S. Fish and Wildlife Service (FWS) Bozeman National Investigational New Animal Drug (INAD) Office (NIO), other public agencies such as state natural resources agencies, the private aquaculture sector, sponsors, and the National Coordinator for Aquaculture NADAs.

PROJECT SUCCESSES - Successes for the IAFWA Project are the (1) identification and retention of committed sponsors for all IAFWA Project drugs, (2) identification of alternate drugs and sponsors to replace sarafloxacin and benzocaine, (3) identification of data requirements for each drug, (4) execution of studies according to acceptable protocols, (5) submissions of technical sections to CVM by its partners mentioned above, and (6) acceptance of these technical section submissions by CVM that should lead to new or supplemental NADA approvals. Currently, all eight IAFWA Project drugs have pharmaceutical or chemical company sponsors, in contrast to only three when the IAFWA Project began in 1994. A first major and tangible success was the broad supplemental NADA approval in 1998 for formalin to control certain fungi on the eggs of all fish and certain external protozoa and monogenetic trematodes on all fish. Other broad approvals are expected for hydrogen peroxide and oxytetracycline (OTC). Limited approvals are expected for chloramine-T, copper sulfate, and florfenicol.

YEAR 7 HIGHLIGHTS

AQUI-S™

An INAD was established for AQUI-S™ at the Bozeman INAD Office after the sponsor made a decision to renew its efforts to gain approval for its original anesthetic formulation

Chloramine-T

All holders of chloramine-T INADs were sent notices in March 2001 that CVM has concerns for the possible carcinogenicity of p-TSA, the marker residue of chloramine-T and will not renew slaughter authorizations (including release of fish) after a certain point (depends upon the date of INAD renewal). This policy will continue until CVM receives new information from the sponsor that addresses CVM's mammalian safety concerns for p-TSA.

CVM has accepted data demonstrating the effectiveness of chloramine-T at a concentration of 12 mg/L administered as a 60-minute bath every other day for three treatments for the control of mortality associated with bacterial gill disease (BGD) in freshwater-reared salmonids.

Copper Sulfate

CVM is requiring no tolerances, regulatory methods, or withdrawal times for fish treated with copper sulfate.

Crop Grouping

Drs William Gingerich, William Hayton, and Guy Stehly presented a morning-long seminar at the Office of Research, Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, Maryland on August 30, 2000 to review the results of efforts to date on crop grouping studies conducted by UMESC.

Florfenicol

A Cooperative Research and Development Agreement (CRADA) between Schering-Plough Animal Health (SPAH) and USGS was signed on April 10, 2001. The in-life phase of a target animal safety study for florfenicol in channel catfish has been completed under that CRADA.

Funding was made available to UMESC and FWS for efficacy data generation on florfenicol under the Multi-State Conservation Grant Program on November 1, 2000.

Hydrogen Peroxide

CVM accepted pivotal efficacy data for treatment of salmonid eggs to control mortalities associated with saprolegniasis and data to control mortality associated with bacterial gill disease (BGD) on all salmonids.

CVM is requiring no tolerances, regulatory methods, or withdrawal times for fish and their eggs treated with hydrogen peroxide.

UMESC has continued to expand its coordination and collaboration to develop additional efficacy data to support the use of hydrogen peroxide by initiating three compassionate INADs. Participation in the three INAD protocols has increased immensely over the past year from 24 INAD units in 2000 to 115 units in 2001.

Negotiations and Contract Coordination

The sponsor, AQUI-S New Zealand LTD., reversed a business decision to reformulate their product. The product to be developed in the United States is the same formulation that the company has approved as a fish anesthetic in several countries.

On October 19, 2000, Axcentive bv signed an amended Cooperative Research and Development Agreement (CRADA) with the U.S. Geological Survey (USGS) to cover studies and cooperation on their chloramine-T product, Halamid™.

Akzo Nobel Chemicals, Inc., the United States representative for the sponsor of chloramine-T, Axcentive bv, is actively engaged in responding to concerns raised by the Center for Veterinary Medicine (CVM) regarding its submission of two genotoxicity studies in the Fall of 2000.

Axcentive bv held a meeting with CVM and UMESC on November 29, 2000 to discuss the development of its environmental assessment on Halamid™.

Pfizer Inc. sold its OTC medicated feed products to Philbro Animal Health (Fort Lee, New Jersey) on September 28, 2000. Philbro Animal Health has expressed an interest in

expanding and extending its NADAs for aquaculture.

The Joint Subcommittee on Aquaculture's (JSA) Aquaculture Effluents Task Force (AETF) met on June 7, 2000 and September 20-21, 2000 to discuss the status of the U.S. Environmental Protection Agency's (EPA) Effluent Guidelines Plan for aquaculture facilities. A white paper related to effluent issues for drugs and chemicals was submitted to EPA on August 24, 2000. A conference call was convened on May 30, 2001 to discuss drug and chemical issues with EPA.

A bill entitled "Minor Animal Species Health and Welfare Act of 2000" was introduced in the U.S. Congress into the House on June 27, 2000 (HR-4780) and into the Senate on October 5, 2000 (S-3169). The Act will facilitate and accelerate the approvals of aquaculture drugs. A revised bill "Minor Use Minor Species Animal Health Act of 2001 was reintroduced into the House on May 24, 2001 (HR-1956) and into the Senate on August 2, 2001 (S-1346).

Oxytetracycline

CVM has accepted data demonstrating the effectiveness of oxytetracycline-medicated feed at 3.7 g OTC/100 lbs fish/day for ten consecutive days for the control of mortality associated with coldwater disease in fingerling coho salmon.

CVM has accepted data demonstrating the effectiveness of oxytetracycline-medicated feed at 3.75 g OTC/100 lbs fish/day for ten consecutive days for the control of mortality associated with columnaris (causative agent *flavobacterium columnare*) in juvenile steelhead trout.

CVM accepted human food safety data for juvenile northern pike and walleye. The data were sufficient to establish a zero withdrawal time in these species.

CVM accepted human food safety data for juvenile coho salmon. The data allow OTC to be used on all juvenile salmonids at any culture temperature with a three-day withdrawal time.

UMESC obtained funding from Federal Aid in Sport Fish Restoration Funds to conduct an environmental assessment for OTC.

DRUG STATUS

Certain label claims are nearing completion (see Tables 1 and 2 for details):

Chloramine-T--mortality from bacterial gill disease on salmonids reared in freshwater
Copper sulfate--*Ichthyophthirius* on catfish in earthen ponds
Florfenicol -- mortality from furunculosis in salmonids (submitted by the sponsor)
Florfenicol - - mortality from environmental septicemia in channel catfish (to be submitted by sponsor)
Formalin--mortality from saprolegniasis on all fish
Hydrogen peroxide--mortality from saprolegniasis on all fish eggs
Hydrogen peroxide--mortality from saprolegniasis on all fish
Hydrogen peroxide--mortality from bacterial gill disease on salmonids reared in freshwater
Oxytetracycline--mortality from systemic columnaris disease in all salmonids
Oxytetracycline--mortality from systemic coldwater disease in all salmonids
Oxytetracycline--otolith marking of all fish by immersion (submitted by the National Research

Support Project Number 7)

All the technical sections (except efficacy) for which the IAFWA Project is responsible will be submitted by 2002 to allow for broader label claims when efficacy data are generated beyond 2002 for chloramine-T, formalin, hydrogen peroxide, and oxytetracycline (see Table 1 for details). The sponsor of florfenicol is completing the technical sections on florfenicol for salmonids and catfish but the Drug Approval Working Group decided not to allow IAFWA Project funds to be expended to extend the label claims to cool and scaled warm water fish. Amendments to broaden initial or existing aquaculture drug approvals will be possible after pivotal and supporting efficacy data are generated and accepted to substantiate label claims beyond those mentioned above (see Tables 2 and 3 for details). At the present time, adequate efficacy data exist mainly for salmonids but are lacking for cool water and warm water fish (see Table 3 for details). This lack of efficacy data jeopardizes the addition of these species to label claims in original or amended NADAs.

New INADs are now in place at NIO to develop efficacy data on florfenicol and AQUI-S™, and Cooperative Research and Development Agreements are also in place with the sponsors for AQUI-S™, copper sulfate, chloramine-T and florfenicol. In the latter half of 2000, new sponsors replaced the original sponsors for chloramine-T and oxytetracycline.

Details of all items in this executive summary can be found in the complete text of the Seventh Annual Report of Progress located on the UMESC website at http://www.umesc.er.usgs.gov. This document will be available on the UMESC website after September 15, 2001.

CHALLENGES - Although the IAFWA Project is producing successes for the development of drugs for public aquaculture, three major factors have hampered efforts to gain broad approvals for all IAFWA Project drugs: (1) loss of drugs from the IAFWA Project, (2) lack of successful efforts to generate required data to demonstrate effectiveness, especially for cool and warm water fish, and (3) additional requirements for original or revised environmental assessments.

ISSUES AND ACTION ITEMS:

1. **Efficacy Issues --** There are three efficacy issues that must be addressed. These include: (a) Label claims for cool and warm water fish cannot be added to many original or amended NADAs because efficacy data are lacking; (b) Continued existence of INADs after IAFWA Project completion is uncertain because INAD holders need to show progress toward approval for remaining label claims or the INAD will be cancelled by CVM; (c) Use of drugs beyond that allowed in approved NADAs (i.e., Extra-Label Use) will be difficult or impossible because certain limitations will restrict their use. These are: (1) a valid veterinarian-client-patient relationship is required, (2) a drug used beyond that allowed on its label must be an approved animal or human drug in the approved formulation and obtained from the legitimate sponsor; (3) Use of an alternative approved drug is possible only if the drug approved for that disease does not work, and (4) Extra-Label Use is very limited for medicated feed applications in aquaculture because no “top coating” of feed with a drug is allowed and producers can use only oxytetracycline and Romet-30 trout and catfish medicated feeds at the present time.

Alternative Actions:

- Accelerate state involvement;
- Determine through CVM what INADs can continue if no progress is being made to complete the efficacy requirements for certain label claims;
- Leave efficacy effort as is and rely on limited Extra Label Use to cover the species and diseases not on the approved label and risk that a number of fish species will go untreated and be lost to production.

Priority Recommendations for Efficacy Studies

The IAFWA Drug Approval Working Group needs both to prioritize drugs and their label claims to facilitate development of efficacy data for broad approvals. Possibilities for these priorities are found mainly in the red blocks of Table 2. Project coordinators are making the following recommendations in priority order based on the likelihood of the successful completion of all technical sections for the following label claims:

1. Hydrogen peroxide - Supporting efficacy data for saprolegniasis on cool and warm water fish
2. Hydrogen peroxide - Additional supporting efficacy data for saprolegniasis on cool and warm water fish eggs
3. Hydrogen peroxide - Pivotal and supporting efficacy data for bacterial gill disease on cool and warm water fish
4. Hydrogen peroxide - Pivotal and additional supporting efficacy data for external columnaris disease on all fish
5. Hydrogen peroxide - Pivotal efficacy data for external parasites on all fish
6. Oxytetracycline - Pivotal and supporting efficacy data for systemic columnaris disease in cool and warm water fish
7. Oxytetracycline – Pivotal efficacy data for *Aeromonas* sp. in cool water fish
8. Chloramine-T – Pivotal and supporting efficacy data for external columnaris disease on all fish
9. Chloramine-T – Pivotal and supporting efficacy data on bacterial gill disease for cool and warm water fish
10. Copper sulfate – Uses of copper sulfate other than for control of *Ichthyophthirius* on all fish in earthen ponds
11. Potassium permanganate – Uses of potassium permanganate other than for *Ichthyophthirius* on fish in earthen ponds

2. Chloramine-T Mammalian Safety Issues --Additional mammalian safety studies on chloramine-T may be required by CVM if the sponsor cannot resolve the issues.

Alternative Actions:

- Identify funding sources from outside of IAFWA Project to fund mammalian safety studies required by CVM (possible sources include FWS, the company sponsor, private aquaculture sector, other granting sources);

- If adequate outside funding sources cannot be identified, stop work on chloramine-T;
- Stop work on other IAFWA Project drugs and redirect funds to required mammalian safety studies.

3. **AQUI-S™ Funding Needs--**Funds are needed to initiate the residue chemistry data on AQUI-S™ for a representative coldwater fish. Completing this technical section will require research efforts beyond the final year of the IAFWA Project.

   Alternative Actions:

   - Redirect existing Crop Grouping Project funds to initiate required research;
   - Rely on other unidentified funding sources;
   - Do not conduct required studies.

Table 1. Status of technical sections (except efficacy) for "all fish" label claims

Project Drug	Product Chemistry	Mammalian Safety	Environmental Safety			Human Food Safety			Target Animal Safety		
	All Fish	All Fish	Cold	Cool	Warm	Cold	Cool	Warm	Cold	Cool	Warm
AQUI-S™											
Chloramine-T											
Copper sulfate (eggs)						NA	NA	NA			
Copper sulfate (fish)											
*Florfenicol (sponsor only)											
Formalin (eggs)											
Formalin (fish)											
Hydrogen peroxide (eggs)											
Hydrogen peroxide (fish)											
Oxytetracycline											
Potassium permanganate (eggs)						NA	NA	NA			
Potassium permanganate (fish)											

Legend

	Work required but not begun
	Data collection underway
	Data submitted to CVM
	Data accepted by CVM
	Prior or amended approval
	Not applicable

*CVM Responses unknown due to proprietary nature of sponsor INAD

Table 2. Status of efficacy technical sections for "all fish" label claims

Project Drug	Disease Indication	Fish Group[1]					
		Cold water fish		Cool water fish		Warm water fish	
		Pivotal	Supporting	Pivotal	Supporting	Pivotal	Supporting
AQUI-S™	Anesthetic						
Chloramine-T	Bacterial gill disease						
	External columnaris disease						
Copper sulfate	Saprolegniasis - eggs						
	Saprolegniasis - fish						
	External columnaris disease						
	Ichthyophthirius						
	Other external parasites						
*Florfenicol	Furunculosis (sponsor)			NA	NA	NA	NA
	Systemic columnaris disease						
	Enteric septicemia (sponsor)	NA	NA	NA	NA		
	Coldwater disease			NA	NA	NA	NA
Formalin	Saprolegniasis - eggs						
	Saprolegniasis - fish						
	External parasites						
Hydrogen peroxide	Saprolegniasis - eggs						
	Saprolegniasis - fish						
	Bacterial gill disease						
	External columnaris disease						
	External parasites						
Oxytetracycline	Systemic columnaris disease						
	Systemic coldwater disease			NA	NA	NA	NA
	Aeromonas sp.						
	Marking otoliths						
Potassium permanganate	Saprolegniasis - eggs						
	Saprolegniasis - fish						
	External columnaris disease						
	Ichthyophthirius						
	Other external parasites						

	Work required but not begun		Data accepted by CVM
	Data collection underway		Prior or expanded approval
	Data submitted to CVM	NA	Not applicable

[1] Designations may represent one or more species within a fish group; * CVM responses unknown due to proprietary nature of sponsor INAD

Table 3. Efficacy data needed for "all fish" label claims

Drug (lead facility)	Disease	Study Type	Fish Group
Chloramine-T (NIO)	Bacterial gill disease	Pivotal/ Supporting	Cool and warm water fish
	External columnaris disease	Pivotal/Supporting	All fish
Copper sulfate (HKD-SNARC)	Saprolegniasis on fish eggs	Supporting	Warm water fish eggs
	Saprolegniasis on fish eggs	Pivotal/Supporting	Cool and cold water fish eggs
	Saprolegniasis on fish	Pivotal/Supporting	All fish
	External columnaris disease	Pivotal/Supporting	All fish
	External parasites (except *Ichthyophthirius*)	Pivotal/Supporting	All fish
Hydrogen peroxide (UMESC)	Saprolegniasis on fish eggs	Pivotal/Supporting	Cool and warm water fish eggs
	Saprolegniasis on fish	Supporting	All fish
	Bacterial gill disease	Pivotal/Supporting	Cool- and warm water fish
	External columnaris disease	Pivotal/Supporting	All fish
	External parasites	Pivotal/Supporting	All fish
Oxytetracycline (NIO)	Systemic columnaris disease	Pivotal/Supporting	Cool and warm water fish
	Systemic cold water and columnaris diseases	Supporting	Additional salmonids
	Aeromonas sp.	Pivotal	Cool water fish
Potassium permanganate (HKD-SNARC)	Saprolegniasis on fish eggs	Pivotal/Supporting	All fish eggs
	Saprolegniasis on fish	Pivotal/Supporting	Cool and cold water fish
	External columnaris disease	Pivotal/Supporting	All fish
	Ichthyophthirius	Pivotal/Supporting	Cool and cold water fish
	Ichthyophthirius	Supporting	Warm water fish
	External parasites (except *Ichthyophthirius*)	Pivotal/Supporting	All fish

Legend:

	HKD-SNARC=Harry K. Dupree Stuttgart National Aquaculture Research Center
	NIO=U.S. Fish and Wildlife Service National INAD Office
	UMESC=Upper Midwest Environmental Sciences Center

TABLE 4. Glossary of acronyms used in this report.

AETF	Aquaculture Effluents Task Force
AVS	Acid Volatile Sulfides
BGD	Bacterial Gill Disease
BRD	Biological Resources Division, U.S. Geological Survey, U.S. Department of the Interior
CRADA	Cooperative Research and Development Agreement
CVM	Center for Veterinary Medicine, Food and Drug Administration, U.S. Department of Health and Human Services
DAWG	Drug Approval Working Group
EA	Environmental Assessment
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration, U.S. Department of Health and Human Services
FOI	Freedom of Information
FWS	Fish and Wildlife Service, U.S. Department of the Interior
HKD-SNARC	Harry K. Dupree Stuttgart National Aquaculture Research Center, Agricultural Research Service, U.S. Department of Agriculture
HPLC	High Performance Liquid Chromatography
INAD	Investigational New Animal Drug exemption
JSA	Joint Subcommittee on Aquaculture
LRP	Low Regulatory Priority
NADA	New Animal Drug Application
NIO	Bozeman National Investigational New Animal Drug Office, U.S. Fish and Wildlife Service, Department of Interior
NRSP-7	National Research Support Project Number 7
OTC	Oxytetracycline
PMF	Public Master File
p-TSA	Para-toluenesulfonamide
UMESC	Upper Midwest Environmental Sciences Center, Biological Resources Division, U.S. Geological Survey, U.S. Department of the Interior
USGS	U.S. Geological Survey, U.S. Department of the Interior

SUMMARY OF PROGRESS BY RESEARCH STUDY PLAN

The following is a summary of the progress made from July 1, 2000 to June 30, 2001 for each of the ten research study plans in the IAFWA Project.

STUDY NO. 1: FORMALIN, EXTENSION OF LABEL FOR USE AS A FUNGICIDE ON FISH AND THEIR EGGS PRODUCED AT PUBLIC AQUACULTURE FACILITIES.

Objectives: To develop suitable efficacy and target animal safety data to extend the current NADA for formalin to include its use to control saprolegniasis on eggs and adults of publicly cultured freshwater fish.

Expected Products: (A) Submission of a technical section on efficacy by UMESC to support an amended NADA for formalin to control or prevent saprolegniasis on all fish eggs. (B) Submission of a technical section on target animal safety for all fish and their eggs. (C) Approval of an amended NADA for formalin to control and prevent saprolegniasis (fungal infections) on all fish eggs and control external parasitic infestations on all fish. (21CFR529.1030, approved supplemental NADA for Western Chemical, Inc., June 18, 1998). (D) Submission of a technical section on efficacy by UMESC to support an amended NADA for formalin to control and prevent saprolegniasis on all cultured freshwater fishes.

Job No. 1: (**Completed**) Coordination of formalin compassionate INAD exemptions and NADA submissions.

Job No. 2: Conduct controlled laboratory studies on a variety of fish species to evaluate the efficacy of formalin to control or prevent saprolegniasis on cultured freshwater fish and their eggs.

> **Progress:** CVM determined that there were insufficient data to support a label claim that formalin was effective in controlling mortalities in cold- and warm water fish infected with saprolegniasis based on data generated under the formalin compassionate INAD exemptions. In response, UMESC drafted a protocol entitled, "Efficacy of formalin for treating saprolegniasis in channel catfish". The protocol was submitted to CVM for formal review and will be used to generate pivotal efficacy data for formalin to control mortalities associated with saprolegniasis on catfish. UMESC is working with the CVM's Office of Research to conduct a pivotal efficacy study on rainbow trout infected with *Saprolegnia*. This study should be initiated early in FY 2002.
>
> **Current Status:** As a result of the decision by CVM, UMESC is working with the CVM's Office of Research to conduct the required pivotal efficacy studies on rainbow trout infected with *Saprolegnia*. The research is being funded and conducted by CVM. No IAFWA Project funds will be expended on these efforts to gain a label claim for control of saprolegniasis on all fish.

Job No. 3: (**Completed**) Conduct target animal safety studies on fish and fish eggs with formalin in support of its extended use as an antifungal agent in public aquaculture.

STUDY NO. 2: OXYTETRACYCLINE, EXPANSION OF FEED ADDITIVE FOR CONTROL OF BACTERIAL DISEASES AND OTOLITH MARKING ON FISH.

Objectives: (1) Extend the feed additive label for treatment of certain bacterial diseases on cool- and warm water fish species of importance to public fish production and to cover marking of fish species not covered by a current approval. (2) Expand the feed additive label for control of flavobacteriosis on cold-, cool-, and warm water fishes.

Expected Products: (A) Approval of an amended NADA for OTC as a marking agent on all fish. (B) Submission by UMESC of NADA technical sections in human food safety and efficacy to support an amended NADA for OTC to control systemic flavobacteriosis for all salmonids below and above 9°C. (C) Submission by UMESC of NADA technical sections in human food safety and efficacy to support an amended NADA for OTC to control mortalities associated with systemic flavobacteriosis in one representative cool- or warm water species. (D) Submission by UMESC of NADA technical section on efficacy to support an amended NADA for OTC to control mortalities associated with bacterial diseases currently on the label in representative cool or warm water species.

Job No. 1: Develop efficacy data or determine if current data on OTC medicated feed are adequate to expand the label.

Progress:

NIO:

$ The NIO received a response from CVM to a pivotal efficacy trial for treatment of steelhead with columnaris disease that stated the study "adequately demonstrates the effectiveness of a treatment regimen of 3.75 g OTC/100 lbs. of fish for ten days to reduce mortalities resulting from systemic columnaris infections in steelhead trout".

$ The NIO received a response from CVM to a pivotal efficacy trial for treatment of coho salmon with coldwater disease that stated the study "adequately demonstrates the effectiveness of a treatment regimen of 3.7 g OTC/100 lbs fish/day for ten consecutive days for the control of mortality associated with coldwater disease in fingerling coho salmon."

UMESC:

$ UMESC analytical chemistry staff continue to analyze feed samples in support of pivotal efficacy studies being conducted under the compassionate INAD for OTC and coordinated by NIO. Before initiation of pivotal efficacy studies with OTC, UMESC analytical chemistry staff should be contacted to coordinate the collection, shipment, and analysis of supporting feed samples.

$ UMESC researchers collaborated with the state of Iowa (Rathbun Research Facility, Moravia, Iowa) to conduct a pivotal efficacy trial to control mortalities associated with columnaris disease in walleye (*Stizostedion vitreum*). The efficacy trials conducted to date were either unsuccessful or inconclusive.

Current Status:

NIO:

$ The NIO is continuing to develop pivotal efficacy data on OTC as the opportunities arise.

UMESC:

$ The UMESC will continue to support pivotal efficacy trials, coordinated by NIO at participating INAD hatcheries, by supplying analytical support to determine OTC in feed until requirements for efficacy are met. UMESC researchers will also continue to collaborate with personnel at the cool and warm water hatcheries (such as Rathbun Research Facility, Iowa) to conduct pivotal efficacy trials to control mortalities from systemic columnaris disease in walleye in the summer and fall of 2001.

Job No. 2: Develop residue chemistry data on OTC in cold-, cool-, and warm water fish.

Progress:

Residue Depletion Studies:

$ Separate marker residue depletion study reports for the cool water fish species northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum*) were submitted to CVM on October 4, 2000. Because the OTC residues in edible fillet tissues in both species were below the current tolerance of 2 ppm immediately after the 10-day OTC treatment, UMESC petitioned CVM to shorten the withdrawal time for OTC in all freshwater fish species.

$ In a letter dated May 15, 2001, the Center for Veterinary Medicine (CVM) stated that residue depletion studies submitted on October 4, 2000 for walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*) fed OTC medicated feed were conducted satisfactorily. Additionally, CVM stated that the data supported a zero withdrawal time in juvenile northern pike fed OTC at the approved dose level for 10 days at water temperatures down to 14°C. For juvenile walleye the data supported a zero withdrawal time at the approved dose level for 10 days at water temperatures down to 16°C.

$ CVM accepted residue chemistry studies on OTC for use on salmonids below 9°C, and established a withdrawal time of three days for juvenile salmonids treated with OTC medicated diet. On March 26, 2001 a request was sent to CVM to extrapolate a withdrawal time for oxytetracycline in all sizes of salmonids based on available residue depletion data. The request included copies of 18 peer reviewed scientific journal articles that included residue data on several salmonid species under different conditions.

OTC Analytical Method in Fish Tissue:

$ On December 8, 2000, CVM sent a letter to UMESC asking for clarification on certain aspects of the OTC analytical method in fish tissue that was used by UMESC to generate residue depletion data in salmonids and selected cool water fish. These data were submitted by UMESC to CVM on July 10, 2000.

Current Status:

Residue Depletion Studies:

$ No additional OTC residue depletion studies are planned.

OTC Analytical Method in Fish Tissue:

$ UMESC personnel are responding to questions from CVM by modifying the standard operating procedures to conform to the agency=s residue chemistry methods procedures format.

Job No. 3: (Assumed Complete) Develop target animal safety data on OTC in cool- and warm water fish.

Progress: No activity in the period from July 1, 2000 to June 30, 2001.

Current Status: Target animal safety studies were completed for the original NADA to cover all cold- and warm water fish. Target animal safety studies conducted according to Good Laboratory Practice regulations will be required for cool water fish, unless there are adequate pivotal and supporting efficacy studies on these additional species to demonstrate that OTC is safe. At this point in the IAFWA Project, only one efficacy study on a cool water species (northern pike) has been accepted by CVM as supporting data for efficacy.

Job No. 4: (**New Job/Assumed Complete**) Submit complete package for a supplemental NADA for the marking of all fish by immersion

Progress: On November 1, 1999, the CVM liaison to NRSP-7 submitted to the Public Master File (PMF) a complete data package for a supplemental NADA for the marking of all fish by immersion to support a future supplemental NADA that will hopefully be submitted by an NADA sponsor. CVM will publish the availability of the data via the Federal Register provided that they are acceptable and a sponsor can then reference the PMF submission for an NADA supplemental approval.

Current Status: The data package for marking is currently under review by CVM. When these data are accepted by CVM, this job will be completed.

Job No. 5: (New Job) Develop an EA of OTC for use in public aquaculture

Progress: At a meeting between CVM, UMESC, NIO, and the National Aquaculture NADA Coordinator on December 14, 2000, CVM reviewers indicated that an EA would have to be completed for OTC before any amendment to the existing OTC label would be possible. This represents a new and unanticipated job for the project. Funding for an OTC EA was subsequently obtained from the Federal Aid in Sport Fish Restoration Reverted Fund account (July 25, 2001). The proposal includes measurement of OTC in water and sediment in and around a hatchery with a typical treatment for a disease episode.

Current Status: A literature search for the EA has begun and the report will be initiated with the completion of the chloramine-T EA.

STUDY NO. 3: COPPER SULFATE APPROVAL TO CONTROL EXTERNAL PROTOZOAN AND METAZOAN PARASITES AND BACTERIAL AND FUNGAL DISEASES OF CULTURED FOOD FISH.

Objectives: To gain approval of copper sulfate as a therapeutant to control external protozoan and metazoan parasites, bacterial, and fungal diseases of cultured food fish.

Expected Products: (A) Technical section submissions for a new NADA for copper sulfate as a microbicide for all fish. (B) Approval of copper sulfate for the control of *Ichthyopthirius* on all fish.

Job No. 1: (**Completed**) Develop research protocols for determining distribution of residual copper in organs and tissues of fish that have been exposed to copper sulfate.

Job No. 2: (**Completed**) Conduct studies of residues of copper in organs and tissues of cultured channel catfish that have been exposed to copper sulfate at therapeutic levels.

Job No. 3: Prepare an EA of the fate and effects of release of treatment water containing copper sulfate.

Progress: A previous submission of the EA has been reviewed by CVM and additional data are needed on the relationship between AVS and copper toxicity.

Current Status: A study of the relationship between AVS and copper toxicity in sediments of channel catfish ponds is underway.

Job No. 4: (**Completed**) Conduct studies of residues of copper in organs and tissues of cultured food fish, other than channel catfish, that have been exposed to copper sulfate at therapeutic levels.

Job No. 5: (**New Job**) (**Assumed Complete**) Prepare an efficacy technical section on copper sulfate and submit to CVM.

Job No. 6: (**New Job**) Prepare a target animal safety technical section on copper sulfate and submit to CVM.

Progress: Data collection phase of Target Animal Safety study on channel catfish is complete.

Current Status: Currently in analysis and audit phase. Anticipate submission of report to CVM by November 1, 2001.

Job No. 7: (**New Job**) (**Assumed Complete**) Prepare an ecological risk assessment of the use of copper sulfate in aquaculture.

Progress: A previous ecological risk assessment submission has been reviewed by CVM and they require additional data on the relationship between AVS and copper toxicity. Revision of Environmental Assessment is nearly complete. Limited additional primary research is required.

Current Status: Anticipate submission of revision to CVM by January 1, 2002.

Job No. 8: (**New Job**) Prepare Freedom of Information (FOI) Summary for copper sulfate

Progress: No activity in the period from July 1, 2000 to June 30, 2001.

Current Status: No activity in the period from July 1, 2000 to June 30, 2001.

STUDY NO. 4: CHLORAMINE-T APPROVAL TO CONTROL BACTERIAL GILL DISEASE ON SALMONIDS AND FLAVOBACTERIOSIS ON COLD-, COOL-, AND WARMWATER FISH SPECIES

Objectives: Develop data on mutagenicity, environmental fate, residue chemistry, efficacy, and target animal safety that satisfy CVM requirements to support the approval of chloramine-T to BGD and external flavobacteriosis on cultured freshwater fish.

Expected Products: (A) Submissions by UMESC of NADA technical sections to support a new NADA for chloramine-T in human food safety (regulatory and confirmatory analytical methods in a variety of fish; marker residue depletion studies in fish), efficacy to external flavobacterial infections, and target animal safety. (B) Submissions of NADA technical sections by the sponsor for product chemistry, mammalian toxicology, and environmental safety.

Job No. 1: Conduct mammalian studies in support of the approval of chloramine-T as a drug.

Progress: Axcentive bv submitted two genotoxicity studies to CVM in November 2000 to assess the potential mammalian toxicology of para-toluene sulfonamide (p-TSA), the marker residue of the Axcentive bv chloramine-T product, Halamid J . Axcentive bv received a response from CVM on March 15, 2001 that indicated the agency had concerns about the two studies. CVM needs to accept the mammalian safety technical section for the agency to establish a tolerance for its marker residue, p-TSA. Establishing a tolerance is an important step in developing the regulatory and confirmatory analytical methods required to complete the human food safety technical section for Halamid J (see No. Job 4 below).

Current Status: Akzo Nobel Chemicals, Inc., the United States representative for the sponsor of Halamid™ is actively engaged in responding to concerns raised by the CVM.

Job No. 2: Environmental fate and effect studies in support of the approval of chloramine-T as a drug.

Progress: A comprehensive review of the public literature on the environmental safety of chloramine-T is in progress at UMESC. This environmental summary will present a detailed discussion of the fate of organic chloramines in general, with emphasis on their likelihood to produce 1) free chlorine, 2) actively chlorinating exchange products, or 3) chlorinated carcinogens. The report will also incorporate chloramine-T use and discharge data from a hatchery survey conducted by UMESC.

Current Status: UMESC is currently preparing an environmental summary of chloramine-T use in public aquaculture based on information available in the literature. It will be submitted to

CVM, who will be requested to place it in the UMESC PMF for chloramine-T.

Job No. 3: Coordination of chloramine-T compassionate INAD exemptions and NADA submissions.

Progress:

NIO Pivotal Efficacy Studies:

$ The NIO was notified on June 30, 2000 by phone (and on July 11 by letter) that the chloramine-T efficacy technical section, for the indication described below, was accepted: *For use to control mortalities of freshwater-reared salmonids caused by bacterial gill disease. Treat fish 1 to 3 times at 12 - 20 mg/L for 1 hour in a static or flow-through treatment system. If fish are treated more than once, treat on consecutive or alternate days.* No additional pivotal efficacy trials have been conducted.

UMESC Pivotal Efficacy Study Protocol Development and Efficacy Testing:

$ A study was conducted to compare the efficacy of chloramine-T to control mortalities associated with external columnaris disease on cultured finfish at the UMESC. Walleye infected with columnaris were treated with 10, 20 and 30 mg/L of chloramine-T for 60 minutes every other day, on three occasions. Fish mortalities were lower in the treatment groups when compared to the control group. However, the external columnaris infection progressed into a systemic infection that compromised the study.

Current Status:

NIO Pivotal Efficacy Studies:

- Pivotal efficacy technical section is considered complete for bacterial gill disease on salmonids in freshwater.

UMESC:

$ Pivotal efficacy trials for external columnaris disease and bacterial gill disease are anticipated to continue in the spring and summer of Project Year 8 for cool and warm water fish. Implementation of the field trials depends on whether disease outbreaks occur at cooperating public and private hatcheries where personnel have volunteered to participate.

Job No. 4: Residue chemistry studies to support the approval of chloramine-T as a drug.

Progress:

- **Determinative Method:** A method developed at the UMESC to determine p-TSA concentrations in edible fillet tissue of three freshwater fish species was submitted to CVM for review. CVM scientists suggested appending the data so that the method would cover all species of freshwater fish cultured at public hatcheries. The request included the following tasks: Task 1) evaluate the method accuracy and precision with channel catfish (*Ictalurus punctatus*) and walleye (*Stizostedion vitreum*) edible fillet tissue fortified with p-TSA at 500 ng/g (0.5X the expected 1000 ng/g tolerance limit for p-

TSA), 1000 ng/g (1X the expected tolerance limit for p-TSA), and 2000 ng/g (2X the expected tolerance limit for p-TSA); Task 2) evaluate the method precision with edible fillet tissue from at least five channel catfish and at least five walleye containing incurred p-TSA after exposing the fish to chloramine-T; and Task 3) test the applicability of the method with p-TSA fortified rainbow trout fillet tissue (1000 ng/g) from three regions of the country. In addition, the method performance was tested with fillet tissue from Atlantic salmon (a cold freshwater or marine species, genera *Salmo*), hybrid striped bass (a scaled warmwater species), and lake trout (a cold freshwater species, genera *Salvelinus*).

Results from Task 1): The method accuracy and precision with channel catfish fillet tissue were within the range of acceptance established by CVM. The method accuracy was 97.4 % with samples fortified at 500 ng/g, 92.6 % with samples fortified at 1000 ng/g, and 91.6 % with samples fortified at 2000 ng/g. The method precision with channel catfish fillet tissue containing incurred p-TSA at a nominal concentration of 1000 ng/g ranged from 0.99 to 5.8 %.

Results from Task 2): The method accuracy and precision with walleye fillet tissue were within the range of acceptance established by CVM. The method accuracy was 90.9 % with samples fortified at 500 ng/g, 91.6 % with samples fortified at 1000 ng/g, and 87.9 % with samples fortified at 2000 ng/g. The method precision with walleye fillet tissue containing incurred p-TSA at a nominal concentration of 1000 ng/g ranged from 0.99 to 9.9 %.

Results from Task 3): For all species, the mean method accuracy ranged from 98.2 % to 101% with samples fortified at 1000 ng/g with method precision ranging from 1.4 to 2.9 %. These data indicate that the method is broadly applicable for the analysis of p-TSA in edible fillet tissue from multiple species of freshwater fish.

- **Marker Residue Depletion – Cold Water Species:** A residue depletion study with a representative cold water fish species was conducted by exposing rainbow trout *Oncorhynchus mykiss* (mean weight 456.5 g) to 20 mg/L of chloramine-T for 60 min on four consecutive days in water at 8 °C (the most aggressive treatment expected for the label). Groups of 18 fish were sampled immediately after the last treatment, then at 1, 3, 6, 12, 24, and 48 hours (19 fish sample group) after the last treatment. Skin-on fillets from each fish were analyzed for p-TSA with a high performance liquid chromatography method. The mean concentration of p-TSA in fillets from fish sampled immediately after the last treatment was 96.4 ng/g and was 73.7 ng/g 48 hours after the last treatment. The p-TSA concentration in the fillet tissue from any individual fish was less than 149 ng/g, less than 15 % of the probable tolerance limit for p-TSA (1000 ng/g) in fish fillets.

- **Marker Residue Depletion – Cool Water Species:** The in-life phase of a marker residue depletion study with a representative cool water species, the yellow perch *(Perca flavescens)*, was completed and fillet tissue samples are being processed to determine concentrations of p-TSA.

Current Status:

- **Determinative Method:** Final reports associated with Tasks 1, 2, and 3 that refine the analytical method for p-TSA in all fish tissues will be submitted to CVM by October 31, 2001.
- **Residue Depletion - Cold Water Species:** A final report associated with the depletion of p-TSA from rainbow trout fillet tissue will be submitted to CVM by October 31, 2001.
- **Residue Depletion - Cool Water Species:** Determination of p-TSA residues in the fillet tissue from exposed yellow perch, *Perca flavescens*, is ongoing and will be completed in early September 2001.
- **Residue Depletion - Warm Water Species:** UMESC will submit a protocol, "Depletion of para-toluenesulfonamide from the edible fillet tissue of hybrid striped bass after exposure to chloramine-T", to CVM for review. Research will begin immediately after the protocol is accepted by CVM and approved by the UMESC Center Director.

Job No. 5: Target animal safety studies in freshwater fish to support the approval of chloramine-T as a drug.

Progress:

$ **NIO Studies on Cold Water Fish:** A study to evaluate the toxicity of chloramine-T on rainbow trout after treatment on three consecutive days has been completed. Results were similar to previous tests (9 experiments) evaluating the effect of exposure on three alternate days.

$ **UMESC Studies on Cool- and Warm Water Fish:** The safety of chloramine-T treatments to fry and fingerlings of five representative species of cool water and warm water fish was determined at UMESC by (1) measuring acute toxicity, (2) identifying apparent sensitive internal and external tissues from exposed fish during gross necropsies, and (3) evaluating feeding behavior.

To evaluate the safety of the maximum treatment regimen likely to be included on an NADA label for chloramine-T, UMESC completed acute toxicity studies in which single daily chloramine-T treatments were administered for four consecutive days in well water at concentrations of 0, 20, 60, 100, or 200 mg/L to fry of northern pike (*Esox lucius*), lake sturgeon (*Acipenser fulvescens*), and walleye (*Stizostedion vitreum*) at 20°C and largemouth bass (*Micropterus salmoides*) fry and channel catfish (*Ictalurus punctatus*) fry at 27°C in Project Year 6. Similar studies were also completed with walleye and channel catfish fingerlings to assess differences in life stage sensitivity, and at three temperatures to assess the effect of exposure temperature on chloramine-T toxicity in Project Year 6.

Because walleye and channel catfish are more sensitive to most water borne therapeutants being tested in the Project, these species were selected as the representative sensitive species to assess histological effects of chloramine-T exposure. UMESC administered single, daily 180-minute chloramine-T treatments on twelve consecutive days in well water at concentrations of 0, 20, 50, and 80 mg/L to walleye at 20°C and channel catfish fingerlings at

27°C. Gross necropsies were performed and histological samples collected before the first exposure, immediately after the last exposure, and seven and fourteen days after the last exposure. No chloramine-T related mortalities were observed during the exposures to assess the histological effects of chloramine-T exposure for walleye, whereas mortalities of about 8% was observed in channel catfish exposed at 80 mg/L.

Based on the findings in the acute toxicity exposures conducted in Project Year 6 and data in the scientific literature, an acute toxicity study was completed in Project Year 7 to assess the effect of water chemistry on the safety of chloramine-T to walleye fingerlings. Chloramine-T exposures at 20 °C in reconstituted soft water at 200 mg/L for 60 min was acutely toxic to all walleye fingerlings; some mortality (~16%) was also observed in walleye exposed to 100 mg/L. No mortalities were observed in the non-medicated control, or exposures to 20, or 60 mg/L. Gills of all fish that died following exposure to chloramine-T were pale; mucus was observed in wet mounts of gills prepared from dead fish. Mucus was generally not observed in wet mounts of gills prepared from control fish nor fish that survived treatment. Exposure to 100 mg/L of chloramine-T for 60 min generally reduced feeding behavior of walleye exposed in reconstituted soft water when compared to controls.

Current Status:

$ **NIO Studies on Cold Water Fish:** A report on the toxicity of chloramine-T to rainbow trout after treatment on three consecutive or alternate days has been submitted to CVM for review.

$ **UMESC Studies on Cool and Warm Water Fish:** Target animal safety exposures at UMESC, with fry and fingerlings of cool and warm water fish are assumed to be complete. Histological tissue samples and blood smears to (1) complete a histological survey of several tissues to determine target tissues affected by chloramine-T and (2) provide histological evidence to document the initial tissue pathology resulting from treatment and the recovery of affected tissues over time have been collected from representative sensitive cool and warm water species (walleye and channel catfish fingerlings). An intra-agency agreement with the Leetown Science Center and UMESC was developed during Project Year 7 to amend a protocol to develop histopathology data. The initial histopathology review by the principal histologist is nearly complete; review of ~10% of the histological specimens by the senior histologist is anticipated by October 2001. The results of this study will be summarized in final report to be submitted to CVM.

STUDY NO. 5: FLORFENICOL APPROVAL AS AN ORAL DRUG TO CONTROL SUSCEPTIBLE SYSTEMIC BACTERIAL DISEASES IN FRESHWATER FISH (NEW TITLE)

Objectives: Develop efficacy, target animal safety, and total residue and metabolism data required for the use of florfenicol to control furunculosis and other susceptible systemic bacterial diseases in freshwater cold-, cool-, and warm water fish.

Expected Products: Development and submission of technical sections to: (A) gain approval of florfenicol as an oral antibacterial to control furunculosis in freshwater salmonids (sponsor); (B) gain approval of florfenicol as an oral antibacterial to control other susceptible systemic bacterial

diseases in freshwater cold-, cool-, and warm water fish.

Change in Status: With the exception of Jobs No. 1 and 4, all work in Study No. 5 has been stopped at the request of the Drug Approval Working Group at their meeting on March 26, 2000 and planned work was diverted to other Project drugs to ensure that there would be some form of approvals for each of those drugs by the end of the project.

Job No. 1: Gain an INAD exemption and support an INAD to evaluate florfenicol as an oral antibacterial to control furunculosis and other susceptible systemic bacterial diseases in freshwater cold, cool, and warm water fish cultured on public hatcheries.

Progress:

NIO:

$ The florfenicol INAD exemption (INAD 10-697) has been granted by CVM. The INAD stipulates a 21day withdrawal period. The first pivotal efficacy study was completed in June 2001 evaluating the effectiveness of florfenicol to control mortality caused by furunculosis in coho salmon.

UMESC:

$ A UMESC scientist was trained by Schering-Plough Animal Health (SPAH) personnel on the analytical method for determination of florfenicol in fish feed. Two UMESC scientists analyzed fortified and unknown fish feed samples for florfenicol that were simultaneously analyzed at SPAH. Based on the results of these analyses, the analytical method was deemed successfully transferred to UMESC and the scientists were certified to conduct analyses of fish feed for florfenicol.

$ Multi-State Conservation Grant Proposal "Analytical Support of Pivotal Efficacy Trials for Use in Public Fisheries" was funded under the Federal Aid in Sport Fish Restoration Act on May 16, 2001. The proposal provides for analyses of fish feed for florfenicol content from pivotal efficacy trials conducted by the Bozeman NIO. In addition, the proposal includes validation of the analytical method on fish feeds not previously completed by the sponsor and used in pivotal trials.

Current Status:

$ **NIO:** Additional efficacy studies are planned for evaluating the effectiveness of florfenicol to control mortality caused by coldwater disease and columnaris disease.

$ **UMESC:** UMESC scientists are currently analyzing fish feeds for pivotal efficacy trials conducted by the Bozeman NIO. In addition, the method has been validated on feeds not previously tested by Schering-Plough Animal Health.

Job No. 2: (Inactive) Conduct residue chemistry studies in freshwater fish to support the use of florfenicol.

Job No. 3: (Inactive) Conduct target animal safety studies with florfenicol in rainbow trout and a cool or warm water species.

Job No. 4: (New Title) Address issues related to antimicrobial resistance in aquaculture drugs to support approval of florfenicol.

Progress: A special session entitled, AAquaculture and drug resistance@, was convened at Aquaculture 2001 on January 25, 2001, Lake Buena Vista, Florida. Topics included (1) biology of antibiotic resistance, (2) antibiotic resistance in the salmonids and channel catfish industries, (3) standardization of susceptibility testing, and (4) the negligible public health risk from antimicrobial us in aquaculture.

Current Status: Data packages on florfenicol from the sponsor are under review by CVM. At the present time, no decision has been made on antimicrobial issues and the use of florfenicol in aquaculture.

Job No. 5: (Inactive) Prepare an EA of the fate and effects of the release of florfenicol.

STUDY NO. 6: A POTASSIUM PERMANGANATE APPROVAL TO CONTROL EXTERNAL PROTOZOA AND METAZOAN PARASITES AND BACTERIAL AND FUNGAL DISEASES OF CULTURED FOOD FISH.

Objectives: Gain approval of potassium permanganate as a therapeutant to control external protozoan and metazoan parasites and bacterial and fungal diseases of cultured food fish.

Expected Products: Submission of all major technical data sections (i.e. product chemistry, mammalian toxicology, human food safety, environmental safety, efficacy and target animal safety) to support an NADA for potassium permanganate as an external microbicide for freshwater fish.

Job No. 1: (Completed) Develop research protocols for determining distribution of residual manganese in organs and tissues of fish exposed to potassium permanganate.

Job No. 2: (Completed) Conduct studies of manganese residues in organs and tissues of cultured channel catfish exposed to potassium permanganate at therapeutic levels.

Job No. 3: Prepare an EA of the fate and effects of release of potassium permanganate treated water.

Progress: Literature evaluation has been largely completed and an assessment of the state of knowledge of the effect of manganese on the receiving environment has been started. Contact has been made with the Department of Environmental Science and Arkansas State University to develop a cooperative research effort to provide the data needed for the EA. Completion of the Environmental Assessment will depend upon funding. If funding is available, the EA can be completed and submitted to CVM by January 1, 2003.

Current Status: Awaiting completion of cooperative agreement with Arkansas State University. If there is no funding or if funding is significantly delayed, the final EA will be delayed.

Job No. 4: (Completed) Conduct studies of manganese residues in organs and tissues of cultured

food fish other than channel catfish exposed to potassium permanganate at therapeutic levels.

Job No. 5: (New Job) Conduct efficacy studies on potassium permanganate.

Progress:

- **HJD-SNARC:** Have shown efficacy in preventing ichthyophthiriasis in channel catfish and tilapia. Efficacy for control of *Ichthyophthirius multifillis* in channel catfish has been demonstrated.

- **UMESC**: UMESC submitted a protocol ("Efficacy of potassium permanganate for treating saprolegniasis in channel catfish") to CVM for formal review. The protocol was accepted by CVM and the non-clinical study evaluating the efficacy of potassium permanganate to control mortalities in channel catfish infected with saprolegnia was completed. The results of the study indicated potassium permanganate treatments administered to saprolegniasis infected channel catfish did not improve the survival of the fish when compared to the saprolegniasis infected control group.

Current Status:

- **HJD-SNARC: A** report for submission to CVM is expected by March 1, 2002.

- **UMESC:** A completion report will be drafted for the study, the study archived and the report submitted to HJD-SNARC. The report is likely to be submitted to the potassium permanganate public master file by HJD-SNARC personnel.

Job No. 6 : (New Job) Conduct target animal safety studies on channel catfish and rainbow trout.

Progress: Target animal safety studies on channel catfish have been completed and data are in audit status. Will initiate target animal safety studies on rainbow trout in spring, 2001.

Current Status: Expect to submit target animal safety data to CVM review before July 1, 2001.

STUDY NO. 7: AQUI-S J APPROVAL AS AN ANESTHETIC AND SEDATIVE FOR FISH (REVISED TITLE)

Objectives: Submission of efficacy, target animal safety, and residue depletion technical data required for the approval of AQUI-S J as an anesthetic/sedative with a short withdrawal time for several species of freshwater fish.

Expected Products: Submission of technical data to gain approval of a short withdrawal time general anesthetic for public aquaculture.

Job No. 1: Develop a compassionate INAD request to evaluate AQUI-S J as an anesthetic/sedative for fish cultured at public hatcheries.

Progress:

NIO Supporting Efficacy Studies:

- The NIO submitted a request to CVM to establish an INAD for AQUI-S™. CVM granted an INAD (INAD 10-541) with the stipulation that there be a 21-day withdrawal period for fish exposed to AQUI-S™. The NIO received a letter from CVM dated November 29, 2000 permitting the use of AQUI-S™ to treat up to 100 million fish with 5 to 34 mg/L AQUI-S™ in a static bath for 1 to 10 minutes.

UMESC Pivotal Efficacy Studies:

$ On November 17, 2000, UMESC submitted a dose-confirmation study protocol entitled "Evaluation of the efficacy of the fish anesthetic AQUI-S J " for formal review by CVM. Recently, work on the AQUI-S™ efficacy studies at UMESC was redirected to critically needed human food safety residue studies.

Current Status: The sponsor, AQUI-S New Zealand LTD., made a decision to investigate the feasibility of reformulating their product. The sponsor has recently decided to market the same formulation that has been approved as a fish anesthetic in several countries rather than to reformulate the product. No supporting or pivotal efficacy studies could be completed until the formulation decision was finalized.

NIO Supporting Efficacy Studies:

$ Efficacy trials will be initiated in calendar year 2001 under the AQUI-S™ INAD. The NIO encourages hatchery personnel to conduct supportive efficacy trials at their hatcheries to evaluate the effectiveness of AQUI-S™@ as a fish anesthetic. Pivotal efficacy trials will be initiated in calendar year 2001 under the AQUI-S™ INAD. The NIO encourages all hatchery personnel to conduct supportive efficacy trials under INAD 10-547 at their hatcheries to evaluate the effectiveness of AQUI-S™ as a fish anesthetic. A proposal using monies from the Wallop-Breaux Federal Aid in Sport Fish Restoration program was funded to support pivotal and supporting efficacy trials on public fish culture facilities.

UMESC Pivotal Efficacy Studies:

$ Work on the AQUI-S™ efficacy studies at UMESC has now been redirected to critically needed human food safety residue studies. The required efficacy studies will be completed by NIO with funds from outside the IAFWA project (see above).

Job No. 2: Conduct residue chemistry studies in freshwater fish to support the use of AQUI-S J .

Progress: The U.S. representative of AQUI-S New Zealand LTD recently learned that a proposal submitted to the U.S. Department of Agriculture was not funded that would have provided residue data to satisfy human food safety requirements for AQUI-S™. A proposal was made to the IAFWA Drug Approval Working Group (DAWG) to redirect all Year 8 funds for efficacy and target animal safety of AQUI-S™ as well as unfinished crop grouping research to satisfy a portion of the human food safety requirements, i.e. determination of the marker residue.

Current Status: At their meeting at Bozeman, MT on August 2-3, 2001, members of the

DAWG verbally agreed to the redirection of Year 8 funds to conduct studies necessary to determine the marker residue of AQUI-S™. Since greater dosages or longer duration of exposure for anesthetics cannot generally be used to maximize tissue residues (anesthesia results in decreased respiration and therefore decreased uptake), CVM will require information on the combination of temperature, dose, and duration resulting in maximum total residues of AQUI-S™. Once these conditions are defined, pilot total residue depletion and definitive residue depletion studies will be conducted to define the marker residue. Although a zero withdrawal time is preferred for AQUI-S™, information on residues with at least a minimal withdrawal time (e.g. 1 or 2 days) should be determined at this time because a tolerance has not been declared and until the studies are conducted, the magnitude of residues is not known. Since all the requirements for the human food safety of AQUI-S™ cannot be met within the time frame or budget of the IAFWA Project, a proposal was submitted for consideration of funding by the Multi-State Conservation Grant Program (MCGP). The MCGP proposal would fund a study required for the development of a determinative analytical method for the marker residue for AQUI-S™. Additional time and funding will be necessary to complete requirements for the human food safety of AQUI-S™ (i.e., development of a confirmatory analytical method for the marker residue of AQUI-S™ and either the zero withdraw time residue concentration of marker residue or a residue depletion study).

Job No. 3: Conduct target animal safety studies with AQUI-S J in representative cold-, cool-, and warm water species.

Progress:

NIO: This work will be initiated in calendar year 2001 with funds from the Wallop-Breaux Federal Aid in Sport Fish Restoration program.

UMESC: Work on the AQUI-S™ target animal safety studies at UMESC has now been redirected to critically needed human food safety residue studies.

Current Status:

NIO: Work has not been initiated on this job.
UMESC: Recently, the sponsor decided to market the same formulation in this country that has been approved as a fish anesthetic in several other countries. However, work on the AQUI-S™ target animal safety studies at UMESC has been redirected to critically needed human food safety residue studies. The required studies in cold water fish will be completed by NIO with funds from outside the IAFWA project. It is not clear how target animal safety studies in cool and warm water fish will be completed.

Job No. 4: (New Title) Monitor mammalian toxicity studies to support the approval of AQUI-S J .

Progress: A proposal for funding was developed by the U.S. representative of AQUI-S™ to address sub chronic toxicity and was submitted to the U. S. Department of Agriculture in January 2001 for consideration of funding. The U.S. representative of AQUI-S™ is actively engaged in discussions with the principal investigators for mammalian safety studies being conducted on the active ingredient of AQUI-S™ by the National Toxicology Program (NTP).

Current Status: USDA did not fund the proposal submitted by the U.S. representative of AQUI-S™. The mammalian safety studies at NTP are scheduled for completion in the spring of 2002.

Job No. 5: Prepare an EA of the fate and effects of the release of AQUI-S J .

Progress: The sponsor in New Zealand completed a biodegradation study of AQUI-S J in freshwater and saltwater and a report is in preparation. The sponsor is expected to submit the report to CVM in the near future.

Current Status: With the studies completed or submitted to date by the sponsor, it is not clear if the IAFWA Project will incur additional costs associated with environmental safety studies to amend the NADA to include the use of AQUI-S J in freshwater pond and raceway applications.

STUDY NO. 8: HYDROGEN PEROXIDE DEVELOPMENT TO CONTROL SAPROLEGNIASIS, EXTERNAL BACTERIAL INFECTIONS, AND EXTERNAL PARASITIC INFESTATIONS OF FRESHWATER FISHES.

Objectives: Develop efficacy and target animal safety data to provide fish culturists with effective, safe treatment regimens for hydrogen peroxide to control saprolegniasis on fish and fish eggs and for controlling external parasitic infestations and mortalities associated with external bacterial infections on freshwater fish.

Expected Products: (A) NADA technical sections submitted by UMESC related to environmental safety, efficacy, and target animal safety to support an NADA approval for hydrogen peroxide to control mortalities associated with external saprolegniasis on at least one salmonid and one cool or warm water fish species. (B) NADA technical sections related to product chemistry will be submitted in cooperation with the drug sponsor, Eka Chemicals, Inc. (C) Assessments of the efficacy of hydrogen peroxide to treat external parasitic infestations and external bacterial infections on fish will be completed by UMESC and submitted to the hydrogen peroxide PMF.

Change in status: Hydrogen peroxide will retain its current Low Regulatory Priority (LRP) status to control and prevent saprolegniasis on fish and fish eggs; however, as a result of a request by Eka Chemicals Inc. (Marietta, GA) in January 1996, an NADA for hydrogen peroxide is being pursued. CVM stated in June 1995 that LRP status would not apply to external antibacterial or parasiticide uses.

Job No. 1: Conduct efficacy studies on the use of hydrogen peroxide to control saprolegniasis on freshwater fish and fish eggs.

Progress:

- **Efficacy on Fish Eggs**: Efficacy data on the use of 500 to 1,000 mg/L of hydrogen peroxide applied for 15 minutes to control mortalities in salmonid eggs infected with saprolegniasis were accepted by CVM on March 17, 2000. CVM requested additional efficacy data at the low end of the proposed hydrogen peroxide dose range (500 mg/L) for treatment of the eggs of cool and warm water fish species. The UMESC is drafting a report that contains additional efficacy data on hydrogen peroxide treatment concentrations of 250, 500, and 1,000 mg/L administered to cool and warm water fish eggs. The data indicated that the 1,000 mg/L concentration was the most efficacious in reducing mortalities in walleye, paddlefish, and white sucker eggs infected with saprolegnia.

- **Efficacy on Fish**: UMESC drafted a protocol entitled "Efficacy of hydrogen peroxide for treating saprolegniasis in channel catfish". The protocol was submitted to CVM for formal review and will be used to generate pivotal efficacy data for hydrogen peroxide to control mortalities associated with saprolegniasis on catfish.

Current Status:

- **Efficacy on Fish Eggs**: UMESC is drafting a report providing additional efficacy data at the low end of the proposed hydrogen peroxide dose range for the eggs of cool and warm water fish. No further laboratory testing of hydrogen peroxide on freshwater fish eggs is planned.

- **Efficacy on Fish**: UMESC is currently conducting pivotal efficacy studies with hydrogen peroxide to control saprolegnia infections on representative cold (rainbow trout) and warm water (channel catfish).

Job No. 2: Conduct efficacy studies on the use of hydrogen peroxide to control external parasitic infestations and external bacterial infections of freshwater fish at public hatcheries.

Progress:

$ **External bacterial infections:** A technical section on the efficacy of hydrogen peroxide to control mortalities of freshwater fish infected with external bacterial infections was submitted to CVM on January 28, 2000. CVM concluded that the efficacy data were acceptable for the use of hydrogen peroxide to control mortalities of freshwater salmonids infected with bacterial gill disease when applied as a static bath at concentrations of 50 mg/L for 60 minutes, or 100 mg/L for 30 minutes, every other day, for three treatments.

$ **External parasitic infestations:** On May 1, 2000 a technical section on the efficacy of hydrogen peroxide to control parasites on freshwater fish was submitted to CVM. Informal communications between UMESC and CVM on this submission indicated CVM considered the data supportive for salmonids, not pivotal.

Current Status:

- $ **External bacterial infections and parasitic infestations:** Trials evaluating the efficacy of hydrogen peroxide to control external bacterial infections and parasitic infestations on cool- and warm water fish are planned for the spring and summer of Project Year 8. The implementation of these trials depends on disease outbreaks occurring at hatcheries where personnel have volunteered to participate in the hydrogen peroxide INAD studies. UMESC encourages hatchery personnel to conduct supportive efficacy trials at their hatcheries to evaluate the effectiveness of hydrogen peroxide to control external bacterial infections or parasitic infestations on fish. These trials will be conducted under the hydrogen peroxide INAD 10-023 administered by the UMESC.

Job No. 3: Conduct target animal safety studies on fish and fish eggs with hydrogen peroxide.

Progress:

- $ **Target Animal Safety Studies with Eggs:** CVM reviewed target animal safety studies conducted on cold, cool, and warm water fish eggs and requested additional data to show an adequate safety margin above the potential NADA upper treatment limit of 1,000 mg/L for 15 min through hatch. UMESC agreed to provide additional safety data on eggs of rainbow trout and one sensitive warm water species, using exposure concentrations between 1,000 and 2,500 mg/L to better delineate the toxicity of hydrogen peroxide.

- $ **Target Animal Safety Studies with Fish:** Target animal safety studies for fish have been completed.

Current Status:

- $ **Target Animal Safety Studies with Eggs:** Study data from the target animal safety studies completed with rainbow trout and paddlefish eggs were discussed with CVM on December 14, 2000. Based on the discussion, summary reports are being prepared to describe the results of the exposures and will be submitted to CVM in Project Year 8. No additional studies are currently planned.

- $ **Target Animal Safety Studies with Fish:** A report documenting the acute and sub-acute toxicity of hydrogen peroxide exposure to fish was submitted to CVM (October 19, 2000) to fulfill the target animal safety technical section requirements for all fish species.

Job Number 4: (New Job) Prepare an EA of hydrogen peroxide use as a waterborne fish therapeutant in public aquaculture.

Progress: On November 29, 2000 an informal meeting was held with CVM environmental staff to determine the review status of the EA for hydrogen peroxide submitted by UMESC on March 14, 2000. After checking its status in their cue, CVM indicated that the agency may send it out for contract review. During a December 14, 2000 meeting, CVM indicated that funds would be available to contract out the review.

Current Status: CVM informed UMESC that the EA went to outside contractors in the summer of 2001.

Job No. 5: (New Job) Develop compassionate INAD for product efficacy

Progress:

The number of participants using the compassionate INAD for hydrogen peroxide increased from 24 in 2000 to 115 in 2001 as a result of the decision by CVM to suspend the compassionate INAD for chloramine-T in November 2001.

- **Fungus on Eggs:** Clinical field trials, conducted under INAD 10-023, to document the efficacy of hydrogen peroxide treatments to control fungus on cool and warm water fish eggs were conducted at seven private, state, or federal hatcheries and research facilities. Trials were completed with eggs of walleye (*Stizostedion vitreum*), paddlefish (*Polyodon spathula*), smallmouth bass (*Micropterus salmoides*) and channel catfish (*Ictalurus punctatus*) using production-type egg incubation systems. Treatments were initiated with non-eyed eggs and continued either until eye-up or until all viable eggs had hatched. Naturally occurring fungus infections were observed on eggs in all trials and the fungal pathogens (*Saprolegnia parasitica* and *Saprolegnia ferax*) were positively identified from egg samples submitted from three hatcheries. Eggs were treated daily for 15 minutes with 0, 250, 500, 750, or 1,000 mg/L of hydrogen peroxide. One trial also compared a 500 mg/L hydrogen peroxide treatment to a 1,667 mg/L formalin treatment.
- **Fungus on Fish:** A clinical field trial to describe the efficacy of hydrogen peroxide treatments to control fungus on coldwater fish was conducted at a state hatchery. The trial was completed with brown trout (*Salmo trutta*) in production-type raceway systems. Naturally occurring fungus infections were observed and the fungal pathogen *Saprolegnia parasitica* was positively identified from gill samples submitted. Fish were treated on alternate days for 30 minutes with hydrogen peroxide.

Current Status:

- **Fungus on Eggs:** In three of the four walleye egg trials, hydrogen peroxide treatments of 500 mg/L either increased the probability of egg hatch or were as effective as physical removal of fungus-infected eggs to control mortalities. When compared to formalin treatment at 1,667 mg/L, the probability of walleye egg eye-up was less for eggs treated with hydrogen peroxide but actual average percent eye-up was only about 1.9 to 2.6% less. Fungus was observed in all control egg jars, but was virtually eliminated from walleye and channel catfish eggs treated with either formalin or hydrogen peroxide. Although hydrogen peroxide treatments reduced the number of smallmouth bass eggs removed from treatment units due to fungal infection, no significant differences were observed between the mean percent hatch versus untreated eggs. From the limited paddlefish data collected, hydrogen peroxide treatment, without continuous egg movement (rolling) from fertilization through hatch, was not as effective as rolling of eggs from fertilization through hatch to increase the probability of egg hatch. Reports summarizing the clinical trials are being prepared for submission to CVM in Project Year 8.
- **Fungus on Fish**: In the brown trout fish efficacy trial, cumulative mortality was lowest

in fish treated at 75 mg/L (3 fish) and highest in the untreated control (10 fish). A summary report will be prepared and submitted to CVM in Project Year 8.

Job Number 6: (New Job) Develop FOI for hydrogen peroxide uses.

Progress: : Two Freedom of Information reports have been drafted and are currently in internal review at UMESC. The reports are entitled (1) Freedom of Information Summary: Perox-Aid™ for the Treatment of Fish Diseases on Finfish and (2) Freedom of Information Summary: Perox-Aid™ for the Treatment of Fungal Infections on Finfish Eggs. Both FOI reports summarize target animal safety and efficacy data that has been submitted to CVM.

Current Status: The FOI reports will be submitted to CVM in Project Year 8.

STUDY NO. 9: CROP GROUPING CONCEPT DEVELOPMENT AND EXECUTION OF STUDIES

Objectives: (1) Develop cooperative studies with CVM scientists and university investigators that will result in a reasonable approach to solving problems related to developing extensive residue chemistry data for minor species drug approvals. (2) Develop a course of study to demonstrate similarities and differences in the metabolism and residue chemistry of aquaculture drugs by a broad range of cultured freshwater fish.

Expected Products: Demonstrate to CVM that crop grouping is a viable concept in developing residue chemistry data for waterborne drugs by Year No. 5. Additional work would need to be undertaken to complete crop grouping for an oral drug.

Job No. 1: Development of comparative pharmacokinetics and metabolism data for sarafloxacin\florfenicol in rainbow trout and channel catfish.

Progress: An analytical method for determining florfenicol in plasma of multiple species of fish was validated in channel catfish, lake sturgeon, lake trout, rainbow trout, and hybrid striped bass. Method accuracy and precision were within the range of acceptance established by FDA for methods validation.

Current Status: Validation of the method will also be conducted with yellow perch and no problems are anticipated. Because of the unavailability of radiolabeled florfenicol and the unanticipated need for human food safety data for AQUI-S^{TM}, this Job has been terminated and all effort redirected to conduct AQUI-S^{TM} residue studies.

Job No. 2: Development of comparative pharmacokinetics and metabolism data for florfenicol in phylogenetically diverse aquaculture species.

Progress: Although an analytical method has been validated in multiple species of fish and a protocol has been approved for the pharmacokinetic studies, progress has been slow because of commitments of personnel on studies directly related to approval studies on Project compounds.

Because of the unavailability of radiolabeled florfenicol and the unanticipated need for human food safety data for AQUI-S™, this Job has been terminated and all effort redirected to conduct AQUI-S™ residue studies.

Current Status: Pharmacokinetic studies for florfenicol in diverse species of fish have been terminated and will not be conducted.

Job No. 3: (Completed) Develop comparative pharmacokinetics and metabolism data for benzocaine in rainbow trout and channel catfish.

Progress: A full day seminar was held at the CVM Office of Research, Laurel, MD on August 30, 2000 to discuss the results of efforts to date on crop grouping studies conducted at UMESC. Dr. William Gingerich presented an overview of the concept of crop grouping; Dr. William Hayton of the Ohio State University presented work on compartmental and physiologically based pharmacokinetic modeling studies on benzocaine, conducted by graduate students under his direction; and Dr. Guy Stehly presented work conducted at UMESC on initial modeling efforts with florfenicol.

Current Status: Based on the results of the August 30, 2000 meeting and other meetings on the concept of crop grouping, CVM is taking under consideration the concept of crop grouping for aquaculture drugs. Crop grouping is an important concept to the agency and continuing dialog with CVM is expected on this issue. The effort with benzocaine is considered complete.

Job No. 4: (Completed) Develop comparative pharmacokinetics and metabolism data in phylogenetically diverse species to support or refute a crop grouping concept for fish.

Progress: See Job No. 3 above.

Current Status: See Job No. 3 above.

STUDY NO. 10: NEGOTIATIONS AND CONTRACT COORDINATION

Objectives: (1) Ensure that all data required by CVM for approval through NADAs are developed for the eight priority drugs in a timely, logical, and efficient manner. (2) Coordinate the administration of all contracts by CVM's Office of Science to ensure efficiency, timeliness, and acceptability of data to CVM. (3) Track and report the progress of all studies and ensure that they are proceeding toward approval in a timely, logical, and efficient manner. (4) Assemble and submit NADA technical sections for approval by CVM.

Job No. 1: Determine data requirements for approval of each candidate drug.

Progress: Additional efficacy studies are needed for IAFWA Project drugs for at least one label claim for all eight IAFWA Project drugs. UMESC, HKD-SNARC, and FWS are making efforts to fill both pivotal and supporting efficacy data needs, but data are not available to cover all the needs. The implemented efforts to address these needs are as follows: (1) a nationwide initiative entitled "The Shortest Yard" to target mainly private aquaculture sector implemented by National

Aquaculture NADA Coordinator, (2) DAWG to work with National Aquaculture NADA Coordinator to obtain pivotal efficacy data from state agencies, and (3) National Aquaculture NADA Coordinator wrote article for Fish Health Section Newsletter requesting studies from the members, to gain both pivotal and supporting data for IAFWA Project drugs.

Dr. Joan Gotthardt was selected as the Leader of the CVM Aquaculture Drugs Team in July 2000. The establishment of this team is important because aquaculture drug approvals now have greater stature at CVM than before.

Specific Drugs

AQUI-S J (anesthetic)

- STATUS: The sponsor (AQUI-S New Zealand LTD.) is currently proceeding with worldwide drug approval; in June 2001, the sponsor decided to proceed with the old formulation and not develop a new formulation for the U.S. market

- BOTTOM LINE: The late decision of whether to change the formulation of AQUI-S™ delayed data generation on all technical sections and may impact the submission of all technical sections by 2002.

The IAFWA Drug Approval Working Group (DAWG) decided on March 26, 2000 to continue to support current research on an active ingredient in AQUI-S J whose status as a potential carcinogen will not be known until July 2001, but whose sponsor concluded that the active ingredient is safe based on: (1) an understanding of metabolic pathways that support safety, (2) agreement on safety by independent experts, (3) supportive preliminary results of a parallel National Toxicology Program (N.T.P.) study, (4) similar results on a related active ingredient, eugenol, and (5) similar toxicological studies showed no adverse effect.

PROGRESS ON TECHNICAL SECTIONS ON AQUI-S J :

- ***Product Chemistry*** C Accepted elsewhere; no current activity for the United States; The sponsor, AQUI-S New Zealand LTD., recently decided not to change the active ingredient.
- ***Mammalian Safety*** C The sponsor conducted a review of the mammalian safety literature to determine whether to continue with the original active ingredient in light of National Toxicology Program (NTP) studies to test for its potential carcinogenicity that was originally scheduled for completion in July 2001. The sponsor concluded that the active ingredient is safe and presented these conclusions to CVM on November 18, 1999 and decided to proceed with the drug approval in the U.S. based on their assessment of scientific data that the active ingredient is not a carcinogen.
- ***Environmental Safety*** C The sponsor submitted an environmental summary to CVM and has completed an environmental biodegradation study in freshwater and salt water that the sponsor plans to submit to CVM in the near future.
- ***Human Food Safety*** C On July 7, 1999, the sponsor signed a CRADA with UMESC to conduct residue chemistry studies.

- ***Target Animal Safety*** C Preliminary toxicity studies have been completed at UMESC on a variety of fish species. NIO plans to conduct pivotal target animal safety studies on salmonids with outside funds.
- ***Efficacy*** C Preliminary efficacy studies were completed at UMESC on a variety of fish species. CVM granted NIO and INAD (#10-541) to develop supporting data on AQUI-S J with concentrations between 5 to 34 mg/L in a static water bath for 1 to 10 minutes and with a 21-day withdrawal time.

Chloramine-T (external antibacterial)

$ STATUS: Sponsor (Axcentive bv; formerly Akzo Nobel Chemicals, Inc.) committed to INAD/NADA.

$ BOTTOM LINE: All submissions should be completed by 2002 for control of mortalities associated with bacterial gill disease and external columnaris disease on salmonids and all technical sections except efficacy for bacterial gill disease and external columnaris disease on cool- and warmwater fish.

$ On April 25, 2000, the National NADA Coordinator was informed that Akzo Nobel Chemicals, Inc. had sold its chloramine-T product, Halamid J , to two of its employees and the new company's name is Axcentive bv. All contacts, agreements, and timetables will remain the same. CVM was sent a letter on October 23, 2000 to change the sponsorship of INAD #8086.

$ A meeting with CVM was held on October 16-18, 2000 at UMESC, to discuss the draft labels for chloramine-T, in preparation for a December 14, 2000 meeting with CVM to identify any remaining data gaps for the label claims. These draft labels were sent to the persons who responded to the environmental survey last year for their comments and suggestions.

$ A meeting was held on December 14, 2000 with CVM to discuss the draft labels for chloramine-T; to identify any remaining data gaps for the label claims. Residue chemistry data requirements were clarified for an Aall fish@ label claim. Applications of chloramine-T in continuous-flow systems will be verified by NIO under a GLP study.

$ A meeting was held on August 31, 2000 with CVM and B.L. Mitchell, Inc. concerning the potential development of Actamide J , its chloramine-T product. If B.L. Mitchell, Inc. moves forward with any claims, it will be outside of the IAFWA Project since the IAFWA Project has resources to interact with only one sponsor; however, all data generated with public funds under the IAFWA Project would be available in the PMF to any potential sponsor of a chloramine-T product.

$ PROGRESS ON TECHNICAL SECTIONS ON CHLORAMINE-T (HALAMID J):

- ***Product Chemistry*** C The sponsor, Axcentive bv (a 100% daughter company of PNP Holding bv, Barneveld, The Netherlands) has started to develop the product chemistry technical section for Halamid J .
- ***Mammalian Safety*** C Axcentive bv submitted to CVM in November 2000, two genotoxicity studies on the marker residue of Halamid™ that are proprietary to Axcentive bv; CVM has

some mammalian safety concerns that are being addressed by the sponsor.

- ***Environmental Safety*** C An environmental summary by UMESC is underway and is based on discussions at a November 29, 2000 meeting with CVM. The environmental summary developed on public literature and data will be made available to any chloramine-T sponsor in PMF # 5637.
- ***Human Food Safety*** C CVM accepted two residue chemistry studies by UMESC for total residue depletion and metabolism of chloramine-T in rainbow trout; para-toluene sulfonamide (p-TSA) was established as the major metabolite in fish and declared as a marker residue for chloramine-T in juvenile rainbow trout. CVM accepted a simple colorimetric procedure developed by UMESC for use in efficacy studies for determining chloramine-T concentrations in treatment waters.

  UMESC completed work to refine the regulatory (determinative) method for all freshwater fish and is preparing reports for submission to CVM

  UMESC completed research to bridge the proposed analytical method for p-TSA with an outdated, labor intensive method previously used to quantify p-TSA in fish tissue. Data developed with the proposed method for p-TSA were similar to data developed with the outdated method indicating that the two methods were successfully bridged.

  UMESC completed an interagency agreement with CVM=s Office of Research to develop the confirmatory method for p-TSA in fish tissue to satisfy an all fish label claim. This work will be funded with money UMESC originally set aside in another interagency agreement with CVM to conduct work on another drug, benzocaine.

  UMESC completed a marker residue depletion study in rainbow trout and will soon submit a report to CVM. UMESC will soon complete a marker residue depletion study in yellow perch and will submit a protocol for a marker residue depletion study to be conducted in hybrid striped bass.

- ***Target Animal Safety*** C NIO submitted a technical section on the toxicity of chloramine-T to salmonids. UMESC developed data that will soon be submitted to CVM on the toxicity of chloramine-T to multiple species of fish to support an all fish label claim.
- ***Efficacy*** C Efficacy data requirements are met for chloramine-T for control of mortalities associated with bacterial gill disease on salmonids reared in freshwater at 12 to 20 mg/L for one hour. Pivotal efficacy studies are in progress for control of mortalities associated with external columnaris disease on salmonids and cool water fish.

  The following data are needed: (1) pivotal and supporting efficacy data for control of bacterial gill disease on cool and warm water fish and (2) pivotal efficacy studies for control of external columnaris disease on all fish. In a recent commitment, the North Central Regional Aquaculture Center is providing funds to Iowa for pivotal efficacy studies on percids for control of external columnaris disease.

Copper Sulfate (external microbicide)

$ STATUS: Sponsor Phelps Dodge Refining Corporation, has an acceptable product chemistry

technical section.

$ BOTTOM LINE: All submissions should soon be completed for the control of *Ichthyophthirius* sp. on catfish. The claims for control of *Ichthyophthirius* sp. on all fish and other external microbes on all fish would be based on additional efficacy and target animal safety studies that would be completed in 2002, if stakeholders were interested.

$ PROGRESS ON TECHNICAL SECTIONS ON COPPER SULFATE:

- ***Product Chemistry*** C Accepted by CVM from the sponsor, Phelps Dodge Refining Corporation.
- ***Mammalian Safety*** C Accepted by CVM based on a FOI summary written by CVM on March 3, 2000.
- ***Environmental Safety*** C The revised environmental safety technical section for all fish was reviewed by CVM in 2000 and CVM is requiring a study of the relationship between AVS and copper toxicity. A study is underway at HKD-SNARC that will address use of copper sulfate in ponds.
- ***Human Food Safety*** C Accepted by CVM based on FOI written by CVM on March 3, 2000; no tolerances, regulatory methods, or withdrawal times are needed for fish treated with copper sulfate.
- ***Target Animal Safety*** C HKD-SNARC submitted a revised target animal safety technical section for copper sulfate to CVM on January 10, 2000 and received an informal response that limited target animal safety studies that would address histopathology were needed on several species to address insufficient data for an all fish claim. HKD-SNARC performed such a study on channel catfish in the spring 2001.
- ***Efficacy*** C Accepted by CVM for control of *Ichthyopthirius* sp. on all fish.

  HKD-SNARC conducted pivotal efficacy studies to control fungi on catfish eggs. Needed are pivotal efficacy studies to control (1) fungi on cool- and warmwater fish eggs, (2) fungi on all fish, (3) external columnaris disease on all fish, and (4) external parasites (except *Ichthyopthirius* sp.) on all fish

  Supporting efficacy data considered to be sufficient by HKD-SNARC include the control of (1) fungi on all fish, (2) external columnaris disease on all fish, and (3) external parasites (including *Ichthyopthirius* sp.) on all fish. Needed are supporting efficacy studies to control fungi on all fish eggs.

  FOI document preparation for efficacy, target animal safety, and environmental safety for the control of *Ichthyophthirius* sp. on catfish in ponds by copper sulfate is underway at HKD-SNARC.

  When the target animal safety (in progress) and environmental safety (in progress) technical sections are accepted by CVM, the data requirements will be complete for control of *Ichthyophthirius* sp. on catfish in ponds.

<u>Florfenicol (oral antibacterial</u>)

$ STATUS: the sponsor, Schering-Plough Animal Health, recently allowed the development of florfenicol for approval in the United States; approved in Canada in August 1997 to control furunculosis in Atlantic salmon.

$ BOTTOM LINE: Sponsor will continue to develop data for aquaculture approval but the efforts by the IAFWA Project on florfenicol have been redirected at this time by the IAFWA DAWG to other IAFWA Project drugs.

The National NADA Coordinator and representatives from FWS, CVM, and UMESC met with Schering-Plough Animal Health on May 2, 2000 in Union, New Jersey to discuss the details on how to proceed on the development of florfenicol for public and private aquaculture in the United States.

Discussions on the development of florfenicol were held with CVM at the FWS-INAD Coordination meeting in Bozeman, Montana on August 2-3, 2000.

UMESC and FWS submitted Multi-State Grant Conservation Proposals to develop data on florfenicol for use on publicly cultured fish to the International Association of Fish and Wildlife Agencies in September 2000. The President signed the funding for the Multi-State Conservation Grant Program on November 1, 2000. FWS was funded for two years to develop efficacy data and UMESC was funded for one year of their proposal to support generation of the efficacy data by FWS; additional funding beyond one year for UMESC will be dependent on an assessment of the results on the data developed in the first year and the status of the antimicrobial resistance issue for florfenicol. Initiation of work by NIO under Schering-Plough=s INAD to control systemic bacterial diseases in fish began in the summer 2001. The analytical method to detect florfenicol in the feed was reviewed and accepted by Schering-Plough Animal Health.

<u>Formalin (external microbicide)</u>

$ STATUS: Supplemental NADA by Western Chemical Inc. approved on June 18, 1998 for control of certain fungi on the eggs of all fish and certain external protozoa and monogenetic trematodes on all fish.

$ BOTTOM LINE: All submissions should be completed in 2001 for control of mortalities associated with saprolegniasis on all fish.

- Natchez Animal Supply Company submitted a supplemental NADA to CVM on June 5, 2001 for its formalin product, Formalin-F® to control certain fungi on the eggs of all fish and certain external protozoa and monogenetic trematodes on all fish.

$ PROGRESS ON TECHNICAL SECTIONS ON FORMALIN:

- ***Product Chemistry*** C Accepted by CVM.
- ***Mammalian Safety*** C Accepted by CVM.
- ***Environmental Safety*** C Accepted by CVM.
- ***Human Food Safety*** C Accepted by CVM.
- ***Target Animal Safety*** C Accepted by CVM.

- ***Efficacy B*** Fungal disease model developed for efficacy studies in fish by UMESC.

  CVM informally accepted supporting efficacy for control of fungi on salmonids from NIO and UMESC efforts. Plans are underway by CVM and UMESC to perform pivotal efficacy studies in early 2001 for control of mortalities associated with saprolegniasis on rainbow trout and catfish.

Hydrogen peroxide (external microbicide)

$ STATUS: Currently considered as a low regulatory priority drug for use as a fungicide on fish and fish eggs but CVM has encouraged the development of a NADA; human food safety data requirements are met.

$ BOTTOM LINE: All submissions should soon be completed for control of mortalities from saprolegniasis on all fish eggs; by 2002, for control of mortalities from saprolegniasis on all fish, for control of mortalities from external bacterial gill disease and external columnaris disease on salmonids, and for control of parasites on all salmonids.

A meeting was held on October 16-18, 2000 at UMESC to discuss the draft labels for hydrogen peroxide in preparation for a December 14, 2000 meeting with CVM to identify any remaining data gaps for the label claims. These draft labels were sent to the persons who responded to the environmental survey last year for their comments and suggestions.

A meeting was held on December 14, 2000 with CVM to discuss the draft labels for hydrogen peroxide to identify any remaining data gaps for the label claims. Target animal safety and efficacy data requirements were clarified for an all fish label claim. Applications of hydrogen peroxide in continuous-flow systems will be verified by NIO under a GLP study.

$ PROGRESS ON TECHNICAL SECTIONS ON HYDROGEN PEROXIDE:

- ***Product Chemistry*** C Sponsor, Eka Chemicals, Inc., submitted product chemistry technical section on July 12, 1999; additional data are required that the sponsor is committed to provide; revision underway by sponsor.
- ***Mammalian Safety*** C Accepted by CVM. The FOI summary was written by CVM on March 22, 2000.
- ***Environmental Safety*** C A model was developed by UMESC to estimate discharged environmental concentrations based on UMESC hatchery survey and a point source dilution model from the U.S. Geological Survey. UMESC submitted an EA to support an all fish label claim to CVM on March 14, 2000; CVM sent the EA out for review by outside contractors in the summer of 2001.
- ***Human Food Safety*** C Accepted by CVM. The FOI summary was written by CVM on March 22, 2000; no tolerances, regulatory methods, or withdrawal times are needed for fish and their eggs treated with hydrogen peroxide.
- ***Target Animal Safety*** C Target animal safety technical section on all fish eggs was submitted by UMESC to CVM. The safety data were accepted for a dose range of 500 to 1,000 mg/L for the eggs of northern pike, lake sturgeon, and common carp. Safety data did not demonstrate an adequate margin of safety at 1,000 mg/L for eggs of rainbow trout or

eggs of those remaining species that were tested. A target animal safety study on rainbow trout eggs and eggs of the most sensitive warm water species will be completed soon. The target animal safety technical section to support an all fish label claim was submitted to CVM by UMESC on October 19, 2000.

The target animal safety technical section to support an all fish label claim was submitted to CVM by UMESC on October 19, 2000.

- ***Efficacy*** C A fungal disease model was developed by UMESC for efficacy studies with fish.

CVM accepted UMESC efficacy data on March 17, 2000 and October 12, 2000 for the following: (1) a 15-minute treatment at 500 to 1,000 mg/L with hydrogen peroxide to control mortalities associated with saprolegniasis on all salmonid eggs, and (2) a 60-minute treatment at 50 mg/L or a 30-minute treatment at 100 mg/L with hydrogen peroxide to control mortalities associated bacterial gill disease on salmonids reared in freshwater. Supporting data were accepted for control of mortalities associated with external columnaris disease on yellow perch. After reviewing a May 1, 2000 submission, CVM informally indicated that efficacy data are considered supporting for control of parasites on freshwater salmonids.
CVM requested additional data for an all fish egg claim for hydrogen peroxide. UMESC is drafting a report providing additional efficacy data at the low end of the proposed hydrogen peroxide dose range (500 mg/L) for cool and warm water fish. No further laboratory testing of hydrogen peroxide on freshwater fish eggs is planned.

Pivotal efficacy studies have been conducted or are planned by UMESC to support a label claim to control mortalities from saprolegniasis on all fish; supporting data are needed to support a claim for control of mortalities from saprolegniasis on all fish, especially cool water fish.

Pivotal efficacy studies are needed for the control of (1) mortalities from external columnaris disease for all fish and (2) external parasites on all fish.

Supporting efficacy data are needed for control of (1) mortalities from external bacterial gill disease on at least one other cold water fish to allow an all cold water fish claim and at least one cool and on warm water fish, including relevant culture conditions, (2) mortalities from columnaris disease for cold and warm water fish, and (3) external parasites on cool and warm water fish.

Trials evaluating the efficacy of hydrogen peroxide to control external bacterial infections and parasitic infestations on cool- and warm water fish are planned for the spring and summer of Project Year 8. The implementation of these trials depends on disease outbreaks occurring at hatcheries where personnel have volunteered to participate in the hydrogen peroxide INAD studies.

Oxytetracycline (oral antibacterial)

$ STATUS: Currently approved for control of certain bacterial diseases in catfish, salmonids, and lobsters and as a marking agent in Pacific salmon.

$ BOTTOM LINE: All submissions are considered complete for otolith marking on all fish by immersion. By 2002, all submissions should be complete for control of (1) *Aeromonas* sp. in cool water fish, (2) systemic coldwater disease in salmonids, and (3) mortalities associated with systemic columnaris disease in salmonids and walleye.

$ Pfizer Inc., sold its OTC medicated feed products to Philbro Animal Health (Fort Lee, New Jersey) on September 28, 2000. Philbro Animal Health has expressed an interest in expanding and extending its NADAs for aquaculture.

$ A meeting was held on October 16-18, 2000 at UMESC to discuss the draft labels for OTC in preparation for a December 14, 2000 meeting with CVM to identify any remaining data gaps for the label claims. These draft labels were sent to the persons who responded to the environmental survey last year for their comments and suggestions.

$ A meeting was held on December 14, 2000 with CVM to discuss the draft labels for OTC to identify any remaining data gaps for the label claims. Residue chemistry data requirements were clarified for an all fish label claim. An EA will have to be developed for any new label claims. CVM accepted the human food safety technical section for juvenile salmonids at 9 C. and established a three-day withdrawal period.

$ PROGRESS ON TECHNICAL SECTIONS ON OXYTETRACYCLINE:

- ***Product Chemistry*** C Previously accepted by CVM under original NADA from Pfizer, Inc. (now owned by Philbro Animal Health)
- ***Mammalian Safety*** C Previously accepted by CVM under original NADA from Pfizer, Inc. (now owned by Philbro Animal Health)
- ***Environmental Safety*** C Previously accepted by CVM under original NADA from Pfizer, Inc. (now owned by Philbro Animal Health). CVM is requiring a new EA for any new label claims. UMESC received funding for writing the EA from Wallop-Breaux Sport Fish Restoration Funds. The EA is in progress.
- ***Human Food Safety*** C Previously accepted by CVM for certain label claims under original NADA from Pfizer, Inc. for OTC for cold water species above 9° C and warm water species above 16° C. CVM accepted (1) residue chemistry studies submitted by UMESC for use of OTC below the label claim limit of 9° C which established a withdrawal time of three days for juvenile salmonids, (2) residue depletion studies submitted by UMESC for the use of OTC in juvenile cool water species with a zero withdrawal time, (3) an HPLC method developed by UMESC to detect OTC in feed and fish tissue, and (4) a study completed by UMESC bridging the HPLC OTC detection method to the official microbial assay method. UMESC petitioned CVM to shorten the withdrawal time for OTC in all freshwater fish species based on its residue depletion data and the new tolerance of 2 ppm. Efforts on this technical section are considered complete.
- ***Target Animal Safety*** C Previously accepted by CVM under original NADA from Pfizer, Inc. Target animal safety studies conducted according to Good Laboratory Practice regulations will be required for cool water fish, unless there are adequate pivotal and supporting efficacy studies on these additional species to demonstrate that OTC is safe. At this point in the IAFWA Project, only one efficacy study on a cool water species (northern

pike) has been accepted by CVM as supporting data for efficacy.

- ***Efficacy*** C Previously accepted by CVM under original NADA from Pfizer, Inc. for OTC use on catfish, salmonids and lobsters to control certain systemic bacterial diseases. CVM accepted the use of OTC at 3.75 g/ 100 lbs of fish for 10 days as effective in reducing mortalities from (1) systemic columnaris disease in steelhead trout and (2) systemic coldwater disease in fingerling coho salmon. The efficacy technical section developed by UMESC from a data call-in was accepted as supporting data for control of (1) *Aeromonas* sp. in cool water species, and (2) systemic columnaris disease in salmonids. Both pivotal and supporting efficacy data are still needed for control of systemic columnaris disease in cool and warm water fish.

  Several pivotal efficacy studies completed by FWS to control systemic coldwater disease in salmonids were submitted to CVM; however, more studies may still needed.

  Pivotal efficacy data are still needed for control of *Aeromonas* sp. in cool water species. Supporting efficacy data are needed in additional salmonid species to have an all-salmonid claim for systemic coldwater and columnaris diseases. Both pivotal and supporting efficacy data are still needed for control of systemic columnaris disease in cool and warm water fish. Some supplemental efficacy data have been generated on catfish (columnaris disease), hybrid striped bass (strep infections), and tilapia (strep infections).

  UMESC researchers collaborated with the state of Iowa (Rathbun Research Facility, Moravia, Iowa) to conduct a pivotal efficacy trial to control mortalities associated with systemic columnaris disease in walleye (Stizostedion vitreum). The efficacy trial was inconclusive.

Note: On November 1, 1999, the CVM liaison to NRSP-7 submitted to the Public Master File (PMF) a complete data package for a supplemental NADA for the marking of all fish by immersion to support a future supplemental NADA that will hopefully be submitted by an NADA sponsor. CVM will publish the availability of the data via the Federal Register provided that they are acceptable and a sponsor can then reference the PMF submission for an NADA supplemental approval.

Potassium Permanganate (external microbicide)

$ STATUS: The sponsor, Carus Chemical Company, submitted a product chemistry technical section and a request for categorical exclusion for environmental safety; CVM has requested additional data for both technical sections.

$ BOTTOM LINE: All submissions should be completed by 2002 for control of (1) *Ichthyopthirius* sp. on all fish and (2) mortalities from saprolegniasis on catfish.

$ PROGRESS ON TECHNICAL SECTIONS ON POTASSIUM PERMANGANATE:
- ***Product Chemistry*** C Carus Chemical Company submitted a product chemistry technical section for all fish to CVM on December 8, 1998; additional data are still needed.
- ***Mammalian Safety*** CAccepted by CVM.

- ***Environmental Safety*** C Carus Chemical Company submitted a request for a categorical exclusion from an EA for all fish to CVM on February 23, 1998; CVM is requiring an EA. Carus Chemical Company is working on developing a contract for an EA.
- ***Human Food Safety*** C Accepted by CVM.
- ***Target Animal Safety*** C HKD-SNARC completed a target animal safety study on channel catfish (except for histopathology) and plans to conduct a target animal safety on rainbow trout.
- ***Efficacy*** C HKD-SNARC completed pivotal efficacy studies that demonstrated efficacy to prevent *Ichthyophthirius* sp. on channel catfish and tilapia. HKD-SNARC is initiating a pivotal efficacy study for control of *Ichthyophthirius* sp. on channel catfish and a scaled warm water fish species.

  Staff at UMESC have conducted a pivotal efficacy study of potassium permanganate for control of mortalities from saprolegniasis on channel catfish. The study indicated that potassium permanganate was ineffective in controlling mortality in channel catfish from saprolegnia infections.
  Supporting efficacy data are considered to be sufficient by HKD-SNARC for control of (1) mortalities from saprolegniasis on all fish, (2) mortalities from external columnaris disease on all fish, and (3) external parasites (including *Ichthyophthirius* sp.) on all fish. Needed are supporting efficacy studies for control of mortalities from saprolegniasis on all fish eggs.

  HKD-SNARC will decide whether to address adding other label claims.

Sarafloxacin (oral antibacterial)

$ STATUS: Previously, most of the NADA technical sections were submitted by Abbott Laboratories and accepted by CVM for control of enteric septicemia in catfish. However, CDC presented concerns about the use of all fluoroquinolones in animal health because of the perceived potential for developing pathogen resistance to drugs used in humans. It is doubtful that a new NADA on sarafloxacin or any fluoroquinolone will be allowed for aquaculture uses by CVM. In fact, CVM requested on October 31, 2000 that approvals of sarafloxacin and enrofloxacin in poultry be withdrawn.

$ BOTTOM LINE: Sarafloxacin was replaced by florfenicol as the oral antibacterial and model drug for crop grouping research in January 1998 by a unanimous vote of the IAFWA Project stakeholders.

Current Status: Partial or complete NADA technical sections have been submitted for all eight drugs being developed for aquaculture use under the IAFWA Project. The DAWG voted on March 26, 2000 to redirect efforts from florfenicol to other IAFWA Project drugs; however, funding was made available to NIO and UMESC for efficacy data generation on florfenicol under the Multi-State Conservation Grant Program on November 1, 2000.

Job No. 2: Coordinate the administration of contracts.

Progress:

CVM=s Office of Research Interagency Agreement: An interagency (IA) agreement for p-TSA entitled ADevelopment and validation of a procedure for the confirmation of para-toluenesulfonamide in edible fish tissue for all publicly cultured freshwater fish@ (identification number 224-01-7003) was developed between UMESC and CVM=s Office of Research on January 17, 2001.

Amended Cooperative Research and Development Agreement with Axcentive bv: An existing Cooperative Research and Development Agreement was amended by UMESC and Axcentive bv (formerly Akzo-Nobel Chemicals) on October 19, 2000.

Current Status:

CVM Office of Research Interagency Agreement: The interagency agreement on p-TSA will allow direct participation by CVM=s Office of Research in conducting this study for the IAFWA Project. The study is anticipated to be initiated in the summer of 2001. When the data generated under this interagency agreement are accepted by CVM, it is anticipated that the requirements will be satisfied for a broad confirmatory method for the marker residue p-TSA in all publicly raised freshwater fish. Both a determinative method (by UMESC) and a confirmatory method (by CVM=s Office of Research) are required to complete the requirements for a regulatory method.

Amended Cooperative Research and Development Agreement with Axcentive bv. The amended CRADA updates provisions in the original CRADA between UMESC and Akzo-Nobel Chemicals, Inc. and will allow UMESC to work cooperatively with Axcentive bv to develop chloramine-T as a waterborne therapeutant for use in aquaculture.

Job No. 3: Track the progress of all studies and summarize and report the data.

Progress: Major advances were made toward communication and coordination of INAD/NADAs of high priority drugs important to public fish production at a workshop held by NIO in Bozeman, Montana on August 2-3, 2000. Discussions centered particularly on the status of chloramine-T, AQUI-S J , and florfenicol and the general progress of the IAFWA Project.

The DAWG held a meeting in Indianapolis, Indiana on September 15, 2000 to: (1) discuss the progress being made on the IAFWA Project drugs, (2) support the extension of the IAFWA Project until at least 2002, and (3) discuss the new funding proposal on florfenicol. The DAWG also met in Washington, DC on March 15, 2001 to: (1) hear a report from the National Coordinator for Aquaculture New Animal Drug Applications, (2) discuss action items from the 7th Midyear Report of Progress, (3) review funding proposals for Reverted Wallop-Breaux and Multi-State Conservation Grant Program, and (4) determine needs for the final year of the IAFWA Project.

Current Status: Appropriate progress reports have been and will continue to be presented to

the IAFWA Project participants and stakeholders. Continuing efforts will be made to inform the entire aquaculture community of the progress being made on IAFWA Project.

The federal portion of the IAFWA Project has been secured until September 30, 2002 and efforts are being made to add to the 24 states that currently support the extension.

Job No. 4: Assemble and submit NADA packages to FDA for approval.

Progress: From July 1, 2000 to June 30, 2001, IAFWA Project personnel, sponsors, and NRSP-7 Liaison submitted, or were involved in development of four known technical section data packages or major requests to CVM. Some submissions are undisclosable.

AQUI-S J , CVM Responses.

On November 29, 2000, CVM permitted the use of AQUI-S J under NIO=s INAD to treat up to 100 million fish with 5 to 34 mg/L AQUI-S J in a static bath for 1 to 10 minutes.

Chloramine-T Submissions.

Bisinger, E.C. 2000. Genotoxicity studies on chloramine-T (Halamid J). Submitted by sponsor to CVM, Rockville, Maryland, November, 2000.

Chloramine-T, CVM Responses. CVM accepted efficacy data for a 60-minute treatment at 12 to 20 mg/L with chloramine-T, to control mortalities associated with bacterial gill disease of salmonids reared in freshwater.

Copper Sulfate, CVM Responses. CVM required additional data on the relationship between AVS and copper toxicity to complete the EA on copper sulfate.

CVM is requiring no tolerances, regulatory methods, or withdrawal times for fish treated with copper sulfate.

Hydrogen Peroxide Submissions.

Gaikowski, M.P., C. Densmore, J.J. Rach, W.H. Gingerich, and V. Blazer. 2000. Toxicity assessment of hydrogen peroxide to cold-, cool-, and warmwater fish. Volumes I-III. Completion report for study CAP-97-00048-08. Submitted to the CVM, Rockville, Maryland, October 19, 2000. 177 pp.

Hydrogen Peroxide, CVM Responses. CVM accepted pivotal efficacy data for treatment of salmonid eggs to control mortalities associated with saprolegniasis by a 15-minute hydrogen peroxide treatment at 500 mg/L, and a 60-minute treatment at 50 mg/L or 30-minute treatment at 100 mg/L with hydrogen peroxide to control mortalities associated with bacterial gill disease on freshwater salmonids. CVM requested additional efficacy data at 500 mg/L on eggs of cool and warm water species to control mortalities resulting from saprolegniasis.

CVM accepted as supporting, efficacy data to control mortalities associated with external

columnaris disease on infected yellow perch.

CVM requested additional target animal safety data on eggs of rainbow trout and one additional sensitive warm water species in order to satisfy the target animal safety technical section for an all fish eggs claim. CVM is not requiring tolerances, regulatory methods, or withdrawal times for fish and their eggs treated with hydrogen peroxide.

Oxytetracycline, Submissions.

Bernardy, J.A., C. Vue, and M.P. Gaikowski. 2000. Oxytetracycline residue depletion from northern pike fillet tissue. Study completion report submitted to Division of Human Food Safety, Center for Veterinary Medicine, Food and Drug Administration, Rockville, Maryland, October 4, 2000, 701 pp.

Bernardy, J.A., C. Vue, and M.P. Gaikowski. 2000. Oxytetracycline residue depletion from walleye fillet tissue. Study completion report submitted to Division of Human Food Safety, Center for Veterinary Medicine, Food and Drug Administration, Rockville, Maryland, October 4, 2000, 1514 pp.

Oxytetracycline, CVM Response. CVM accepted residue chemistry studies on OTC for use on salmonids below 9°C, and established a withdrawal time of three days for juvenile salmonids treated with OTC medicated diet.

CVM accepted data that a treatment regimen of 3.75 g OTC/100 lbs of fish for ten days is effective in reducing mortalities resulting from systemic columnaris disease in steelhead trout.

On December 8, 2000, CVM sent a letter to UMESC asking for clarification on certain aspects of the OTC analytical method in fish tissue was used by UMESC to generate residue depletion data in salmonids and selected cool water fish. These data were submitted by UMESC to CVM on July 10, 2000.

Current Status: Each technical section submission to CVM and acceptance of that technical section by CVM brings the IAFWA Project closer to approvals. Thus, the submissions of four technical sections, the acceptance of eight data packages, the clarification or request for additional data for four data packages, and the permission to initiate one INAD are major advances toward those approvals. See Study No. 10, Job No. 1 for the status for the technical section submissions on each IAFWA Project drug.

Job No. 5 (New Title) Address national aquaculture issues

Progress: The Joint Subcommittee on Aquaculture's (JSA) formed the Aquaculture Effluents Task Force (AETF) to coordinate and facilitate input of science-based information to assist in the development of national effluent limitation guidelines and standards for aquaculture facilities by the U.S. Environmental Protection Agency (EPA). An EPA Effluent Guidelines Plan could affect the use and approval of all drugs needing EAs and those currently approved for aquaculture use. AETF met on June 7, 2000 and September 20-21, 2000 to discuss the status of the EPA's Effluent Guidelines Plan for aquaculture facilities. A white paper related to effluent

issues for drugs and chemicals was submitted to EPA on August 24, 2000. The AETF met at Aquaculture 2001 (Lake Buena Vista, Florida) on January 21, 2001 to assess progress, pending issues, tasks in progress, and needed follow-up actions. A conference call was convened on May 30, 2001 to discuss drug and chemical issues with EPA.

A bill entitled "Minor Animal Species Health and Welfare Act of 2000" was introduced in the U.S. Congress into the House on June 27, 2000 (HR-4780) and into the Senate on October 5, 2000 (S-3169). The MUMS Act will facilitate and accelerate the approvals of aquaculture drugs. There is a great need for more co-sponsors. The bill includes provisions for early life stages that should help expedite the approvals of aquaculture drugs that are of interest to public and private fish production. A revised bill "Minor Use Minor Species Animal Health Act of 2001" was reintroduced into the House on May 24, 2001 (HR-1956) and into the Senate on August 2, 2001 (S-1346). The MUMS Coalition met on June 22, 2001 to coordinate the legislative effort on the bill, present information to legislative staff, and contact individual congressmen and senators for their support and sponsorship. Letters were written to follow-up on the contacts.

A special session entitled, "Aquaculture and drug resistance" was convened at Aquaculture 2001 on January 25, 2001, Lake Buena Vista, Florida. Topics included (1) biology of antibiotic resistance, (2) antibiotic resistance in the salmonids and channel catfish industries, (3) standardization of susceptibility testing, and (4) the negligible public health risk from antimicrobial us in aquaculture.

Current Status: Progress is being made toward gaining approval of the MUMS legislation, resolving the issues with EPA on aquaculture effluents, and addressing the antimicrobial resistance issues.

APPENDIX I: PRODUCTS OF THE PROJECT AAPPROVAL OF DRUGS FOR PUBLIC FISH PRODUCTION@ IN THE SEVENTH MIDYEAR REPORTING PERIOD

NADA SUBMISSIONS

Bernardy, J.A., C. Vue, and M.P. Gaikowski. 2000. Oxytetracycline residue depletion from northern pike fillet tissue. Study completion report submitted to Division of Human Food Safety, Center for Veterinary Medicine, Food and Drug Administration, Rockville, Maryland, October 6, 2000, 701 pp.

Bernardy, J.A., C. Vue, and M.P. Gaikowski. 2000. Oxytetracycline residue depletion from walleye fillet tissue. Study completion report submitted to Division of Human Food Safety, Center for Veterinary Medicine, Food and Drug Administration, Rockville, Maryland, October 6, 2000, 1514 pp.

Bisinger, E.C. 2000. Genotoxicity studies on chloramine-T (Halamid J). Submitted by sponsor to CVM, Rockville, Maryland, November 2000.

Gaikowski, M.P., C. Densmore, J.J. Rach, W.H. Gingerich, and V. Blazer. 2000. Toxicity assessment of hydrogen peroxide to cold-, cool-, and warm water fish. Volumes I-III. Completion report for study CAP-97-00048-08. Submitted to the CVM, Rockville, Maryland, October 19, 2000. 177 pp.

STUDY PROTOCOLS

Griffin, B.R., A. Darwish and D.L. Straus. 2000. Copper sulfate target animal safety. Experiment Number HKD-SNARC Cu-1. Submitted to CVM, Rockville, Maryland, September, 2000.

Meinertz, J.R. 2000. Depletion of para-toluenesulfonamide from the edible fillet tissue of rainbow trout after exposure to chloramine-T. UMESC study protocol CAP-00-RBT-03. Approved by the UMESC Center Director, August 25, 2000. 15 pp.

Meinertz, J.R. 2000. Depletion of para-toluenesulfonamide from the edible fillet tissue of rainbow trout after exposure to chloramine-T. UMESC study protocol CAP-01-YEP-04. Approved by the UMESC Center Director May 11, 2001. 16 pp .

Stehly, G.R. 2000. Comparative plasma pharmacokinetics of florfenicol in multiple species of fish to support the concept of crop grouping. UMESC study protocol CAP-99-00087-04. Approved by the UMESC Center Director, November 6, 2000. 36 pp

Stehly, G.R. 2001. Validation Data for the p-TSA determinative method in fish edible fillet tissue: precision and accuracy of the method in rainbow trout from different regions of the country, lake trout, Atlantic salmon, and hybrid striped bass. UMESC study protocol CAP-01-PTSA-03. Approved by the UMESC Center Director, January 30, 2001. 30 pp.

PUBLICATIONS

Huggett, D.B., D. Schlenk and B.R. Griffin. In Press. Toxicity of copper in an oxic stream sediment receiving aquaculture effluent. Chemosphere Accepted August 1, 2000.

Meinertz, J.R., M.P. Gaikowski, G.R. Stehly, W.H. Gingerich, and J.A. Evered. 2001. Oxytetracycline depletion from skin-on fillet tissue of coho salmon fed oxytetracycline medicated feed in freshwater at temperatures less than 9°C. Aquaculture 198, 29-39.

Meinertz, J.R., G.R. Stehly, W.H. Gingerich, and S.L. Greseth. In Press. Performance of a proposed determinative method for p-TSA in rainbow trout fillet tissue and bridging the proposed method with a method for total chloramine-T residues in rainbow trout fillet tissue. Accepted for publication, April 26, 2001, in Journal of AOAC International.

Rach J.J., M.P. Gaikowski, and R.T. Ramsay. 2000. Efficacy of hydrogen peroxide to control mortalities associated with bacterial gill disease infections on hatchery reared salmonids. Journal of Aquatic Animal Health 12:119-127.

Schlenk, D., W.C. Colley, A. El-Alfy, R. Kirby and B.R. Griffin. Effects of the oxidant potassium permanganate on the expression of gill metallothionein mRMA and its relationship to sublethal whole animal endpoints in channel catfish. Toxicological Sciences 54:177-182.

Schnick, R.A. 2001. International harmonization of antimicrobial sensitivity determination for aquaculture drugs. Aquaculture (3-4):277-288.

Schnick, R.A. In press. Aquaculture chemicals. Chapter in Kirk-Othmer Encyclopedia. John Wiley & Sons, Inc., New York, New York.

Straus, D.L. and B.R. Griffin. 2000. Prevention of and initial infestation of *Ichthyophthirius multifiliis* in channel catfish and blue tilapia by potassium permanganate treatment. The North American Journal of Aquaculture 63:14-18.

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Gingerich, W.H., G.R. Stehly, V.K. Dawson, M.P. Gaikowski, J.R. Meinertz, J.J. Rach, R.A. Schnick, and B.R. Griffin. 2000. Approval of drugs for public fish production: sixth annual report of progress [performance period: July 1, 1999 to June 30, 2000]. Biological Resources Division, USGS, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin. August 21, 2000. 54 pp.

Schnick, R.A. 2000. 2000 annual report of the AFS Task Force on Fishery Chemicals. Submitted to the Governing Board and AFS President, Christine Moffitt, Bethesda, Maryland. July 21, 2000. 4 pp.

Schnick, R.A., C.E. Eirkson, and others. 2000. Aquaculture effluents containing drugs and chemicals. Submitted to the Joint Subcommittee on Aquaculture, Aquaculture Effluents Task Force, for transmittal to the U.S. Environmental Protection Agency. August 24, 2000. 8 pp.

Schnick, R.A. 2000. Executive summary of the Federal-State Aquaculture Drug Approval Partnership Project on September 15, 2000. Submitted to the International Association of Fish and Wildlife Agencies. September 15, 2000. 3 pp.

Schnick, R.A. 2000. Minutes to meeting on Actamide J with the Center for Veterinary Medicine and B.L. Mitchell, Inc. Submitted to B.L. Mitchell, Inc., sponsor of Actamide J. September 29, 2000. 6 pp.

Schnick, R.A. 2000. Status of the technical sections for a New Animal Drug Application (NADA) for Halamid J (as of November 1, 2000). Submitted to Axcentive bv (The Netherlands), sponsor of Halamid J. November 1, 2000. 1 pp.

Schnick, R.A. 2000. National Coordinator for Aquaculture New Animal Drug Applications (NADAs). Sixth mid-year report of activities, May 15, 2000 to November 9, 2000. Submitted to Ted Batterson, North Central Regional Aquaculture Center, East Lansing, Michigan. November 10, 2000. 23 pp.

Schnick, R.A. 2000. Confidential: Minutes to meeting on the EA of Halamid J with the Center for Veterinary Medicine. Submitted to Akzo Nobel Chemicals, Inc., U.S. representative for Halamid J sponsor, Axcentive bv. December 19, 2000. 7 pp.

Schnick, R.A. 2000. Efficacy data needed for IAFWA drugs. Submitted to the IAFWA Drug Approval Working Group. December 22, 2000. 1 pp.

Schnick, R.A. 2001. Report of Drug Approval Working Group Meeting. Submitted to Mike Gibson, Chair, Drug Approval Working Group. March 19, 2001. 5 pp.

CONTRACT PROPOSALS

Garling, D.L., R.A. Schnick, W.H. Gingerich, V.K. Dawson, and G.R. Stehly. 2001. Oral antibacterials for cool and warm water fish to help diversify small to medium sized farms. Proposal submitted to Initiative for Future Agriculture and Food Systems, U.S. Department of Agriculture, Washington, DC. April 19, 2001. 24 pp. plus Vitae (14 pp.), Conflict of Interest (5 pp.), Budget (18 pp.), Current and Pending Support (4 pp.), Assurance Statements (2 pp.), National Environmental Policy Act Exclusions Form (2 pp.), and Letters (3 pp.)

Gingerich, W.H., G.R. Stehly, V.K. Dawson, M.P. Gaikowski, J.R. Meinertz, and J.J. Rach. 2000. Florfenicol as an antibacterial for cool and warm water fish: a Multi-State Grant Conservation Proposal. Submitted to the International Association of Fish and Wildlife Agencies. September 8, 2000. 55 pp. (Partially funded, November 1, 2000.)

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Stehly, G.R., and W.L. Gingerich. 2001. Development and Validation of a Determinative Analytical Method for the Marker Residue of AQUI-S, a Fish Anesthetic for Public Fish Facilities and

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Gingerich, W.H. 2000. Update on the Federal/State Aquaculture Drug Approval Project. Annual Meeting of the International Association of Fish and Wildlife Agencies. Indianapolis, Indiana. September 15, 2000.

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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.

Rach, J.J., M.P. Gaikowski, and C. A Perkins. 2001. Hydrogen peroxide a potential broad spectrum therapeutant for treatment of fish diseases. Aquaculture 2001. Orlando, FL. January 21-25

Schnick, R.A. 2000. Overview of NADA Coordinator activities. USFWS-INAD Coordination Workshop, Bozeman, Montana, August 2-3, 2000.

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Stehly, G.R. Overview of the crop grouping approach to broad drug approvals under the Federal/State Aquaculture Drug Approval Partnership Project, research conducted on benzocaine at The Ohio State University, and where and how the approach might be applied to other aquaculture drugs, Center for Veterinary Medicine, Laurel, Maryland, August 30, 2000.

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