Sixth Annual Report of Progress

Performance Period: July 1, 1999 to June 30, 2000

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 six years and is into the second year of a three-year extension to 2002. During the course of the IAFWA Project, substantial progress has been made toward aquaculture drug approvals in every study plan. Currently all eight drugs have pharmaceutical sponsors, in contrast to only three sponsors when the IAFWA Project began in 1994.

SUCCESSES --Substantial New Animal Drug Application (NADA) packages of partial or complete technical sections have been submitted to the Center for Veterinary Medicine (CVM) to support original or supplemental approvals for IAFWA Project drugs. These packages were the results of six years of focused efforts by the Upper Midwest Environmental Sciences Center (UMESC, formerly the Upper Mississippi Science Center), the Harry K. Dupree Stuttgart National Aquaculture Research Center (HKD-SNARC, formerly the Fish Farming Experimental Laboratory), the U.S. Fish and Wildlife Service (FWS) Bozeman National Investigational New Animal Drug Office (NIO), other public agencies such as state natural resources agencies, private aquaculture sector, sponsors, and the National Coordinator for Aquaculture NADAs (National NADA Coordinator). The first success was a supplemental NADA approval for **formalin** to control certain fungi on the eggs of all fish and certain external protozoa and monogenetic trematodes on all fish.

As of June 30, 2000, almost all technical sections had been prepared and either submitted to CVM or are in preparation for submission to CVM to support: (1) **copper sulfate** to control *Ichthyopthirius* on catfish; (2) **formalin** to control mortalities from saprolegniasis on all fish; (3) **hydrogen peroxide** to control mortalities associated with saprolegniasis on all fish eggs; and (4) **oxytetracycline** for the otolith marking of all fish by immersion. Human food safety data was submitted on all salmonids below 9°C and additional human food safety data will be submitted by the end of calender year 2000 to support the use of **oxytetracycline** on all cool water fish. In addition to IAFWA Project submissions, the sponsor for the IAFWA Project drug **florfenicol** has submitted technical sections to support an initial claim to control mortalities associated with furunculosis in cultured salmonids. Details on the status of all the technical sections for each drug can be found in Study Plan No. 10, Job 1.

The IAFWA Drug Approval Working Group (DAWG) and a majority of the original IAFWA Project stakeholders supported the extension of the IAFWA Project for at least three additional years (to 2002) to develop data to support the broad approvals of drugs as originally envisioned in the IAFWA Project proposal. All the participants in the IAFWA Project will continue to work diligently for the development of technical section submissions to help gain approvals for all IAFWA Project drugs. In particular, UMESC will continue to generate data in crop grouping and data in human food safety and target animal safety technical sections with the expectation that a data base will be available to allow for the eventual broad approvals for all the IAFWA Project drugs. Broad drug approvals will be possible after sufficient pivotal and supporting efficacy data are available to substantiate efficacy label claims.

CHALLENGES AND PROBLEMS --While the IAFWA Project has produced and continues to produce marked successes for the development of drugs for public aquaculture, three major factors (loss of drugs from the Project, additional efficacy efforts, and environmental assessments) have negatively impacted its overall efforts to gain broad approvals for all IAWFA Project drugs. In addition, IAFWA Project coordinators were recently informed by CVM that prevention label claims are not possible to obtain at this time. Also, the IAFWA Project drugs need Freedom of Information (FOI) summaries for human food safety, target animal safety, and efficacy, data requirements that were not known or anticipated when the IAFWA Project began.

Loss of two IAFWA Project Drugs: First, the IAFWA Project as well as CVM expended considerable fiscal and personnel resources to develop data for one drug, benzocaine, early in the IAFWA Project. Benzocaine was abandoned when it became clear that (1) no sponsor was interested in developing the drug, (2) no funding was available for two required, expensive mammalian safety studies, and (3) the withdrawal period would likely be a longer than expected. In addition, the new, important IAFWA Project oral drug, sarafloxacin, was removed from consideration for approval in aquaculture after nearly all of the work for an initial approval had been completed by the sponsor and UMESC and the data packages submitted to CVM. While the IAFWA Project has been successful in finding willing sponsors for replacement drugs (AQUI-S™ and florfenicol), four of the original five years were lost to the development of those drugs, funds were expended on certain products that will never be approved, and supporting data developed for the abandoned drugs will not be used. Now it is possible that the IAFWA Project may not complete technical sections for approval of florfenicol because efforts have been redirected to other IAFWA Project drugs because of the antimicrobial resistance issue and the possibility that the data for approval would not be developed by 2002.

Major Efficacy Efforts Second, efficacy data (both pivotal and supporting) anticipated under the existing compassionate Investigational New Animal Drug (INAD) exemptions have not been generated to produce the broad label claims originally envisioned in the initial proposal. As a consequence, UMESC and HKD-SNARC have undertaken work beyond their original work plans to develop the required efficacy data necessary for approvals. Examples of such efforts are found in the attempts by UMESC to provide analytical methods development and direct staff support to conduct pivotal efficacy studies on cool and warm water hatcheries for chloramine-T and oxytetracycline; to develop, coordinate, and monitor hydrogen peroxide efficacy trials under UMESC's INAD; to develop quality data criteria for efficacy studies; to conduct data call-ins for formalin, oxytetracycline, and chloramine-T; to analyze and summarize the information from the data call-ins on formalin, oxytetracycline, and chloramine-T; to develop and submit efficacy technical sections for oxytetracycline and chloramine-T based on the data call-ins; and for HKD-SNARC to conduct pivotal efficacy trials for copper sulfate and potassium permanganate to control *Ichthyopthirius* on channel catfish. These efforts have constituted additional unanticipated expenses to the IAFWA Project. While the research is required to gain broad approvals of the drugs, the unanticipated effort in these areas have taken away from the effort by UMESC and HKD-SNARC to complete the original project goals of developing human food safety and target animal safety data for all the IAFWA Project drugs.

Environmental Assessments. Third, an issue has recently developed that also negatively

impacts efforts to achieve all the goals of the original IAFWA Project. Two sponsor companies, Eka Chemicals, Inc. (hydrogen peroxide) and Axcentive (formerly Akzo Nobel Chemicals, Inc. for chloramine-T) have informed the IAFWA Project coordinators that they are unwilling to expend additional corporate funds to support studies or other drug approval activities for their products other than what has already been expended. The IAFWA Project coordinators had always counted on having access to adequate mammalian toxicology and environmental fate and effects data from the sponsors as a means of controlling IAFWA Project costs. The decisions by these sponsors have forced the IAFWA Project coordinators to take on additional and necessary work outside the scope of the original project to develop environmental assessments for both chloramine-T and hydrogen peroxide. Moreover, if CVM finds that the data in the environmental assessments are inadequate to meet their data requirements, additional environmental fate and effects data will need to be generated requiring additional funds to be spent by the IAFWA Project.

Taken together, these three issues have impacted the breadth of work that can be accomplished within the scope of the original IAFWA Project. A decision about the future direction of research taken and work objectives to be accomplished within the IAFWA Project in the remaining two years was addressed by the IAFWA DAWG at its meeting in Chicago on March 26, 2000. At that meeting, the DAWG redirected funds and work plan activities for the time being from florfenicol to other IAFWA Project drugs. This decision was made to ensure that the other seven drugs did gain some form of approval by 2002.

Acronyms used in this report

BGD	Bacterial Gill Disease
BRD	Biological Resources Division, U.S. Geological Survey, U.S. Department of the Interior
CDC	Centers for Disease Control and Prevention, Food and Drug Administration, U.S. Department of Health and Human Services
CVM	Center for Veterinary Medicine, Food and Drug Administration, U.S. Department of Health and Human Services
DAWG	Drug Approval Working Group, International Association of Fish and Wildlife Agencies
EA	Environmental Assessment
FDA	Food and Drug Administration, U.S. Department of Health and Human Services
FWS	Fish and Wildlife Service, U.S. Department of the Interior
GRAS	Generally Regarded As Safe
HKD-SNARC	Harry K. Dupree Stuttgart National Aquaculture Research Center, Agricultural Research Service, U.S. Department of Agriculture
HPLC	High Performance Liquid Chromatography
IAFWA	International Association of Fish and Wildlife Agencies
INAD	Investigational New Animal Drug exemption
JSA	Joint Subcommittee on Aquaculture
LRP	Low Regulatory Priority
NADA	New Animal Drug Application
NCTR	National Center for Toxicological Research, Food and Drug Administration, U.S. Department of Health and Human Services
NIO	Bozeman National Investigational New Animal Drug Office, Fish and Wildlife Service, U.S. Department of the Interior
NRSP-7	National Research Support Project Number 7
OTC	Oxytetracycline
PBPK	Physiologically Based Pharmacokinetic Model
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

HIGHLIGHTS:

(July 1, 1999 to June 30, 2000)

AQUI-S™ (general anesthetic):

A CRADA was developed between UMESC and the sponsor for AQUI-S™. The CRADA will allow UMESC to work collaboratively with the sponsor to conduct total residue depletion studies in a representative coldwater fish. In addition, UMESC will perform target animal safety and pivotal efficacy studies that will allow the broadest label claims.

The sponsor, AQUI-S New Zealand LTD obtained a 21-day withdrawal period for AQUI-S™ from CVM and NIO has applied for the same under their INAD.

The IAFWA DAWG decided to continue the support of research on AQUI-S™ on March 26, 2000.

Chloramine-T (external microbicide):

An amended CRADA is in the process of being implemented between UMESC and the sponsor (Axcentive, formerly Akzo Nobel Chemicals Inc.). The amended CRADA will allow mutagenicity studies to be conducted and paid for by the sponsor and UMESC to incorporate critical environmental safety data into the environmental assessment that was already developed by, and proprietary to, Axcentive.

CVM accepted the efficacy technical section as complete for chloramine-T to control bacterial gill disease on freshwater-reared salmonids at 12-20 mg/L for three times on consecutive or alternate days. This decision was based on a revised efficacy technical section from compassionate INADs submitted by UMESC on January 10, 2000 and on additional efficacy data submitted by NIO on April 17, 2000.

CVM accepted pivotal efficacy data that indicate that prolonged intermittent therapy with chloramine-T at 20 mg/L was effective in preventing bacterial gill disease in tiger muskellunge; however, CVM is not expected to approve this use when the environmental assessment is reviewed.

UMESC and NIO have completed target animal safety studies on cold, cool and warm water fish and reports are being drafted for submission to CVM. The results indicate that chloramine-T is safe for use on all fishes at the highest efficacious concentration of 20 mg/L.

Akzo Nobel Chemicals, Inc. (now Axcentive) announced that they will not expend any additional corporate funds to develop chloramine-T as a therapeutant for aquaculture; however, the company will provide an acceptable product chemistry technical section and meet their commitments in the amended CRADA when it is finalized.

Copper Sulfate (external microbicide):

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 to address histopathology requirements were needed on catfish, trout, and salmon to obtain an all fish claim. HKD-SNARC is in the process on performing such a study on channel catfish.

Crop Grouping

The experimental phase of the Crop Grouping research on the model waterborne drug benzocaine being conducted at Ohio State University is complete and a final report is in preparation. Preliminary results indicate that there are more similarities than differences in the manner in which diverse groups of fish handle benzocaine.

Florfenicol (oral antibacterial):

The IAFWA DAWG voted to redirect funds and efforts from florfenicol to efforts toward approvals of other IAFWA Project drugs at its meeting on March 26, 2000.

UMESC has completed preliminary validation of the method used to analyze florfenicol in fish feeds and is prepared to support pivotal efficacy studies in a variety of fish species.

Formalin (external microbicide):

CVM's Office of Research is conducting a pivotal efficacy study on rainbow trout to control saprolegniasis with formalin.

Hydrogen Peroxide (external microbicide):

CVM accepted a label claim that a 15-minute treatment at 500 mg/L with hydrogen peroxide controlled mortalities associated with saprolegniasis on all salmonid eggs. CVM required additional efficacy data that were subsequently developed by UMESC on the eggs of one warm water species to better delineate the efficacy of hydrogen peroxide for the same label claim for cool water and warm water fish eggs.

Pivotal efficacy studies were completed and submitted by UMESC to support the approval of hydrogen peroxide to control mortalities resulting from bacterial gill disease in cold water fish (January 28, 2000) and to control parasitic infestations on selected fish (May 1, 2000).

UMESC is coordinating efficacy trials on public and private hatcheries under the UMESC INAD to collect pivotal and supporting efficacy data to support a label claim for hydrogen peroxide to control (1) mortalities associated with external bacterial infections and (2) external parasite infestations on all fish.

An environmental assessment for hydrogen peroxide was submitted by UMESC to CVM on March 14, 2000.

CVM reviewed target animal safety studies conducted on fish eggs and did not agree with UMESC's assessment that hydrogen peroxide administered at 1,000 mg/L for 15 minutes through hatch was safe for all fish species. Additional target animal safety studies on fish eggs were completed by UMESC to better delineate the toxicity of hydrogen peroxide and the final report is in review.

Eka Chemicals, Inc., announced that they will not expend additional corporate funds to develop a NADA for therapeutic uses of hydrogen peroxide for aquaculture but that they will allow development of efficacy data under their INAD and will provide an acceptable product chemistry technical section.

Negotiations and Contract Coordination:

No tolerances, regulatory methods, or withdrawal times are needed for fin fish treated with copper sulfate or for fin fish and their eggs treated with hydrogen peroxide. FOI summaries containing these rulings were written by CVM on March 3, 2000 and March 22, 2000, respectively.

Schering-Plough Animal Health submitted a data package to CVM on September 30, 1999 to support the approval of florfenicol to control furunculosis in all salmonids.

Eka Chemicals Inc. submitted the product chemistry technical section for hydrogen peroxide to CVM on July 12, 1999 and received comments from CVM that will require additional information from the sponsor.

A survey of drug use was used to write an environmental assessment for hydrogen peroxide, and will be used to write environmental assessments for the IAFWA Project drugs AQUI-S™, chloramine-T, florfenicol, hydrogen peroxide, and potassium permanganate.

A white paper was sent on December 20, 1999 to CDC to address the agency's concerns about potential antimicrobial resistance resulting from drug use in aquaculture.

The Minor Animal Species Health and Welfare Act of 2000 (national legislation to improve the ability to gain approvals for aquaculture drugs) was introduced into the House of Representatives on June 27, 2000 and plans are underway to introduce the Bill into the U.S. Senate in early September 2000.

A white paper was sent to EPA on August 24, 2000 to address potential national effluent limitation guidelines and standards for aquaculture drugs and chemicals.

Oxytetracycline (oral antibacterial/bath marking agent):

A residue depletion study was conducted with walleye fed medicated diet by UMESC in collaboration with personnel at the Rathbun Hatchery, Iowa, and a report has been written. A second report for a residue depletion study after OTC treatment (top dressed and mixed ration

feeds) in northern pike is completed. UMESC submitted a report "Validation of an HPLC method for oxytetracycline in coho salmon and northern pike fillet tissue" to CVM on July 10, 2000.

The NIO submitted three efficacy reports on OTC (control of columnaris and coldwater diseases in salmonids) to CVM on June 12, July 14, and August 18, 2000, and is preparing a final report of a study on columnaris disease for submission to CVM.

The CVM liaison to NRSP-7 submitted a complete package for a supplemental NADA for the marking of all fish with OTC by immersion to CVM on November 1, 1999. The package is currently under review by CVM.

Potassium permanganate (external microbicide):

Research by HKD-SNARC on the efficacy of potassium permanganate to prevent ichthyopthiriasis infestations in channel catfish is complete and a manuscript has been accepted for publication. Results indicate that the drug was effective. A current study is underway to determine the efficacy of potassium permanganate in eliminating established ichthyopthiriasis on catfish.

SUMMARY OF PROGRESS BY RESEARCH STUDY PLAN

A summary follows on the progress made during the period from July 1, 1999 to June 30, 2000 for each of the ten research study plans in the IAFWA Project.

STUDY NO. 1: EXTENSION OF FORMALIN 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 New Animal Drug Application (NADA) for formalin to include its use to control fungus (i.e., saprolegniasis) on eggs and adults of publicly cultured freshwater fish.

Expected Products: (A) Submission of a technical section on efficacy to support an amended NADA for formalin to control saprolegniasis on all fish eggs; (B) Submission of a technical section on target animal safety for all fish and their eggs; © Approval of an amended NADA for formalin to control saprolegniasis (fungal infections) on all fish eggs and control external parasitic infestations on all fish by the end of Project Year No. 3. (21CFR529.1030, approved supplemental NADA for Western Chemical, Inc., June 18, 1998). (D) Submission of a technical section on efficacy to support an amended NADA for formalin to control saprolegniasis on all cultured freshwater fishes.

Job No. 1: (Completed) Coordination of formalin compassionate Investigational New Animal Drug (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 saprolegniasis on cultured freshwater fish and their eggs.

Progress: After full review of the INAD efficacy data generated as a result of the data call-in for formalin, CVM determined that there were insufficient data to support a label claim that formalin was effective in controlling mortalities resulting from saprolegniasis on cold water and warm water fish.

Current Status: CVM has granted a supplemental NADA for the use of formalin to control saprolegniasis on all fish eggs and control of external parasites on all fish. As a result of the review controlling saprolegniasis on fish 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 this effort 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: EXPANSION OF OXYTETRACYCLINE (OTC) 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 oxytetracycline as a marking agent on all fish. (B) Submission of NADA technical sections in human food safety and efficacy to support an amended NADA for oxytetracycline to control systemic flavobacteriosis for all salmonids below and above 9˚C. (C) Submission of NADA technical sections in human food safety and efficacy to support an amended NADA for oxytetracycline to control mortalities associated with systemic flavobacteriosis in one representative cool or warm water species. (D) Submission of NADA technical section on efficacy to support an amended NADA for oxytetracycline 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 oxytetracycline medicated feed are adequate to expand the label.

Progress: One data requirement for conducting pivotal efficacy studies is an analytical method that is acceptable to CVM for quantifying OTC in feed. An analytical method developed by UMESC scientists allows analysis of OTC in pelleted diet in support of pivotal efficacy studies. Feed samples have been analyzed in support of pivotal efficacy studies being conducted under the compassionate INAD for OTC and coordinated by the U.S. Fish and Wildlife Service (FWS) Bozeman National Investigational New Animal Drug Office (NIO). Before initiation of pivotal efficacy studies with OTC, UMESC should be contacted to coordinate the collection, shipment, and analysis of supporting feed samples.

A lack of any efficacy data on cool water fish species prompted UMESC to collaborate with the state of Iowa to conduct a pivotal efficacy trial to control mortalities associated with columnaris disease in walleye at the Rathbun Research Facility in Moravia, IA. Attempts to produce fish diseased with systemic columnaris pathogens were unsuccessful in 1999 but will be attempted in 2000.

The CVM indicated that a lack of adequate controls limits the conclusions that can be drawn on efficacy against *A. hydrophila* in northern pike. The CVM response was in a letter dated Feb. 1, 2000 in reference to a submission of efficacy data from a data call-in submitted by UMESC in January 1999.

The NIO submitted the following final study reports to CVM for review on June 12, July 14, and August 18, 2000, respectively: (1) The efficacy of oxytetracycline-medicated feed to control mortality of juvenile steelhead trout *Oncorhynchus mykiss* caused by columnaris, (causative agent *Flavobacterium columnare),* (2) The efficacy of oxytetracycline-medicated feed to control mortality of juvenile steelhead trout *Oncorhynchus mykiss* caused by columnaris (causative agent *Flavobacterium columnare),* and (3) The efficacy of oxytetracycline-medicated feed to control mortality of fingerling coho salmon *Oncorhynchus kitusch* caused by coldwater disease (causative agent *Flavobacterium psychrophilum*).

The NIO successfully completed a study entitled “The efficacy of oxytetracycline-medicated feed to control mortality of juvenile steelhead trout *Oncorhynchus mykiss* caused by columnaris (causative agent Flavobacterium columnare)". The final study report is being written and will be submitted to CVM in 2000. The NIO also conducted two additional efficacy studies (coldwater disease in steelhead trout and columnaris disease in striped bass) that did not result in appropriate data for approval (spontaneous recovery and loose snake plugged the incoming water line, respectively).

Current status: The UMESC will continue to support pivotal efficacy trials coordinated by the NIO at participating INAD hatcheries by supplying analytical support to determine OTC in feed until requirements for efficacy are met. UMESC scientists have been collaborating with personnel at the Rathbun Research Facility (Iowa) to conduct a pivotal efficacy trial to control mortalities from systemic columnaris disease in walleye. The efficacy trials will be conducted under Bozeman's INAD in the summer and fall of 2000. UMESC scientists investigated the possibility of making an argument for the use of oxytetracycline for *A. hydrophila* in northern pike based on other scientific evidence. Because of a paucity of data on oxytetracycline concentrations in blood of northern pike and of minimum inhibitory concentrations of oxytetracycline for *A. hydrophila*, it was not possible to include northern pike on the oxytetracycline label. Pivotal efficacy data for *A. hydrophila* will need to be generated to include northern pike on the oxytetracycline label.

The NIO submitted three efficacy reports to CVM and is preparing another study into a final report for submission to CVM. The NIO is continuing to develop pivotal efficacy data on OTC as the opportunities arise.

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

Progress: A residue depletion study for OTC in walleye was conducted at the Rathbun Research Facility, Moravia, Iowa. Walleye were fed a diet top dressed with OTC for 10 d at approximately 89 mg/kg (slightly greater than the maximum dose allowed for salmonids or catfish). Tissue samples were collected at three times during the treatment and eight times after the termination of treatment and the edible fillet samples were analyzed for oxytetracycline concentrations by a method previously validated at UMESC.

A report on the validation of the OTC analytical method in coho salmon and northern pike edible fillet tissue was submitted to CVM on July 12, 2000. Another report for a residue depletion study after OTC treatment (top dressed and mixed ration feeds) in northern pike is complete and ready for submission to CVM.

Current status: Marker residue depletion studies of OTC in northern pike and walleye will be submitted to CVM in the fall of 2000 Because the OTC residues in edible fillet tissues in both walleye and northern pike were below the tolerance of 2 ppm immediately after the 10-day OTC treatment, an argument will be made to include all freshwater fish species for a shortened withdrawal time based on these residue depletion studies.

Job No. 3: **(Assumed Complete)** Develop target animal safety data on oxytetracycline in cool and

warm water fish.

Progress: No activity July 1, 1999 to June 30, 2000.

Current Status: No additional studies are expected to be required to support OTC target animal safety technical section guidelines.

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

Progress: The CVM liaison to NRSP-7 submitted a complete package for a supplemental NADA for the marking of all fish by immersion to CVM on November 1, 1999.

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

STUDY NO. 3: APPROVAL OF COPPER SULFATE 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: **(Assumed Complete)** Prepare an environmental assessment (EA) of the fate and effects of release of treatment water containing copper sulfate.

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 efficacy technical section on copper sulfate and submit to CVM.

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

Progress: A report entitled "Animal safety data for therapeutic use of copper sulfate on food fish"

was submitted to CVM on November 24, 1997. In their response of April 13, 1999, CVM asked that the report be revised to include additional information. A revised report entitled "Target animal safety of copper sulfate as a disease therapeutant for cultured freshwater fish" was submitted January 10, 2000, as a supplement to the November 24, 1997 report.

Current Status: A critique of all submissions was informally provided by CVM that suggested a need for additional target animal safety studies on catfish, trout, and salmon that concentrated on histopathology if a label claim for all fish is desired. A protocol has been prepared and submitted to CVM for review and work on the study will begin when the approved protocol is received. A decision was made to do the target animal safety study on catfish first and then complete the other studies on trout and salmon after an initial approval was obtained.

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

Progress: An environmental assessment for copper sulfate was previously prepared and submitted to CVM in Project Year 5.

Current Status: The Ecological Risk Assessment is currently in review by CVM.

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

Progress: Sample documents were received to guide preparation of the Freedom of Information Summary (FOI) for copper sulfate. A draft FOI for copper sulfate was prepared.

Current Status: A preliminary draft FOI has been completed by HKD-SNARC and is in revision. When the FOI is accepted by CVM, this job will be complete.

STUDY NUMBER 4: APPROVAL OF CHLORAMINE-T TO CONTROL BACTERIAL GILL DISEASE ON SALMONIDS AND FLAVOBACTERIOSIS ON COLD, COOL, AND WARM WATER 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 control bacterial gill disease (BGD) and external flavobacteriosis on cultured freshwater fish.

Expected Products: (A) Submissions 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: At a January 25, 1999 meeting between CVM, the National NADA Coordinator, the UMESC IAFWA Project Coordinator, and the product sponsor (Akzo Nobel Chemicals, Inc., now Axcentive), CVM indicated that they are requesting two genotoxicity studies (approximate total cost for both of $45,000) to further assess the potential mammalian toxicology of para-toluene sulfonamide (p-TSA), the marker residue of Axcentive chloramine-T product. The studies are the mouse lymphoma assay with and without activation and the mouse micronucleus assay. The sponsor agreed to conduct these studies with funds left over from those they previously contributed to UMESC for drug approval research on chloramine-T under an existing Cooperative Research and Development Agreement (CRADA). If the tests are positive, additional and more costly mammalian toxicity testing would be required. If the tests are negative, no additional mammalian safety testing would be required and CVM should be able to establish a tolerance for the marker residue, p-TSA. Establishment of a tolerance is an important step in developing the regulatory and confirmatory analytical methods required to complete the human food safety technical section for chloramine-T.

Current Status: Axcentive has consented to amend the original CRADA with UMESC that will allow UMESC to transfer back a portion of the money originally given to UMESC by the forerunner of Axcentive. The money was used by the company to conduct the required mammalian toxicity studies before the CRADA was finalized. The original CRADA was amended to the satisfaction of the original sponsor and UMESC and received acceptance by the Private Industry Council in USGS. The studies were completed in early 2000 and need to be submitted to CVM for review.

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

Progress: The existing CRADA with Akzo Nobel Chemicals, Inc. was amended to allow UMESC to conduct an environmental assessment (EA) on chloramine-T with data from a hatchery survey developed by UMESC, data from the public literature and critical environmental safety data from the sponsor's proprietary files. The CRADA needed to be revised to reflect the name change (to Axcentive) resulting from a management buy out. The EA will be based on the maximal use concentration of 20 mg/L that has been suggested by participants in the chloramine-T compassionate INAD. The EA will be submitted to the sponsor who will then submit it to CVM.

Current Status: UMESC is conducting a comprehensive review of public literature of chloramine-T as part of the environmental assessment. In addition to the literature survey, UMESC conducted an assessment of chloramine-T use among aquaculture facilities as part of an environmental assessment of chloramine-T. Development of the draft environmental assessment for chloramine-T is expected to be time consuming since free chlorine, other chlorine exchange products (chloramines, organohalogens), and the degradate p-TSA also have to be considered in the assessment, as well as the parent chloramine-T.

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

Progress:

Pivotal Efficacy Study Protocol Development and Efficacy Testing: A UMESC protocol was completed and will be used to evaluate the efficacy of chloramine-T to control mortalities associated with external flavobacteriosis on cultured cool and warm water fish at selected fish hatcheries. The work is being conducted to ensure that chloramine-T will also be available for use on cool and warm water fish.

Data Call-In: CVM notified UMESC and the NIO of the status of studies submitted in the chloramine data call-in on July 29, 1998. CVM provisionally accepted data generated by the Pennsylvania Boat and Fish Commission (Benner Springs Hatchery) that supported the claim that chloramine-T was effective in preventing BGD in tiger muskellunge when used intermittently over a three-week period. Although CVM accepted the efficacy data on tiger muskellunge, CVM is not expected to approve this use when the environmental assessment is reviewed.

In the pivotal efficacy studies to control mortalities resulting from BGD in salmonids , CVM noted that the data tables previously submitted in completion reports for three FWS pivotal efficacy trials of chloramine-T seemed to be in error as were summary tables provided by UMESC. CVM asked that the tables be revised and the reports be resubmitted. The tables were revised by personnel at the NIO to more accurately represent the results obtained during those hatchery trials. An amended summary report based on the three revised FWS pivotal efficacy completion reports was submitted by UMESC to CVM on January 10, 2000. One comment from CVM on the FWS reports was that there were no data at the low end (5 mg/L) of the dose range and requested that additional data be provided to define the low end of the efficacy for chloramine-T if 5 mg/L is to be on the approved label.

To complete the data call-in, NIO submitted the following final study report to CVM requesting a formal review on April 17, 2000: Efficacy of chloramine-T to control mortality caused by bacterial gill disease in fingerling rainbow trout *Oncorhynchus mykiss.* Chloramine-T was administered as a static bath solution (12 mg/L) for 1 h on three alternate days. The NIO also submitted the following final study reports to CVM requesting no formal review at this time on January 20 and April 15, 2000, respectively: (1) Efficacy of chloramine-T to control mortality caused by bacterial gill disease in juvenile rainbow trout *Oncorhynchus mykiss*. Chloramine-T was administered as a bath solution (20 mg/L) for 1 h on three alternate days and (2) Efficacy of chloramine-T to control mortality caused by bacterial gill disease in fingerling Apache trout *Oncorhynchus apache*. Chloramine-T was administered as a bath solution (20 mg/L) for 1 h on three alternate days.

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 mortality of freshwater-reared salmonids caused by bacterial gill disease. Treat fish 1 to 3 times at 12 - 20 mg/L for 1 h in a static or flow-through treatment system. If fish are treated more than once, treat on consecutive or alternate days.

Bridging Study to Support Chloramine-T Pivotal Efficacy Studies: A simple, rapid, and accurate Hach kit analytical procedure to quantify chloramine-T concentrations in water during pivotal efficacy trials, developed by UMESC, was accepted by CVM in 1998. However, CVM suggested that the report be amended by establishing acceptable time limits for holding collected

water samples before analysis because of the effect of organic material on the performance of the method. In side-by-side comparisons of the two methods analyzing timed samples of a 14.5 mg/L solution of chloramine-T containing elevated concentrations of organic material, the two methods were in good agreement for up to 2 hours after the water samples were analyzed. The Hach kit method slightly overestimated the chloramine-T concentration relative to the HPLC method after 2 hours. However, the analysis values achieved by the two methods were still within 7 % agreement even after a 6-hour holding period.

Current Status--All the information from the call-in of data from compassionate INAD's for chloramine-T to control mortality from BGD in fish were resubmitted to CVM on January 10, 2000 for review.

CVM has accepted as complete the chloramine-T efficacy technical section for use to control mortality of freshwater-reared salmonids caused by BGD. Other studies are underway or have been submitted.

Research to develop a simple, rapid, and accurate analytical procedure to quantify chloramine-T concentrations in water during pivotal efficacy trials is complete. A report for a study requested by CVM describing the effects of time after sample water collection is being prepared for submission to CVM.

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

Progress: UMESC completed validation of the analytical method to determine p-TSA (the marker residue of chloramine-T) concentrations in a cold water fish species (rainbow trout). Method accuracy and precision data were within the range of acceptance established by FDA. UMESC also 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.

Current Status: UMESC is continuing research to validate the regulatory analytical method for p-TSA in cool and warm water fish species. After completing the method validation, UMESC will conduct residue depletion studies with cold, cool, and warm water fish species. In preparation for these studies, UMESC initiated a dialogue with CVM to plan the impending residue depletion study in a cold water fish.

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

Progress:

Cool and warm water fish--The safety of chloramine-T treatments to selected cool water and warm water fry and fingerlings was assessed 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 administered single daily chloramine-T treatments for four consecutive days in well water at concentrations of 0, 20, 60, 100, or 200 mg/L to individual groups of test fish. Tests have been completed in cool water fry (northern pike *Esox lucius*, lake sturgeon *Acipenser fulvescens*, and walleye *Stizostedion vitreum*) at 20˚C and in representative warm water fry (largemouth bass *Micropterus salmoides* and channel catfish *Ictalurus punctatus*) at 27˚C. Because of their sensitivity to chloramine-T, additional tests were completed in walleye and channel catfish fry and fingerlings to determine sensitivity at different life stages, exposure temperatures and prolonged daily exposure durations.

Acute toxicity: Chloramine-T at 200 mg/L was acutely toxic to northern pike fry at 20˚C and channel catfish at 27˚C whereas no chloramine-T related mortalities occurred in lake sturgeon or walleye fry tested at 20˚C or largemouth bass fry tested at 27˚C. Chloramine-T toxicity to walleye and channel catfish fry was influenced by exposure time and temperature. Walleye fingerling were more sensitive to chloramine-T exposure than were walleye fry, but in similar exposures, channel catfish fingerlings were less sensitive than channel catfish fry.

Gross necropsy observations: 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. Pale, translucent livers were commonly observed after treatment with chloramine-T, especially for northern pike that died during or shortly after exposure to 200 mg/L.

Feeding behavior: Exposure to 100 and 200 mg/L of chloramine-T for 60 min treatment regimens generally reduced feeding behavior of surviving walleye and channel catfish when compared to controls. Feeding behavior of surviving northern pike, lake sturgeon, and largemouth bass was generally not altered by exposure to chloramine-T.

Cold water fish--Target animal safety studies with rainbow trout are currently underway at the NIO. The safety of chloramine-T treatments to rainbow trout fry, fingerling, and juvenile life stages were assessed by (1) measuring acute toxicity and (2) evaluating pathologies and histological changes in select fish tissue (i.e., gill, skin, eye, kidney, and liver) caused by exposure to chloramine-T.

Acute toxicity: All eleven studies have been completed when test fish were exposed to chloramine-T on three alternate days. Study results show that (1) chloramine-T is safe at the proposed maximum efficacious therapeutic concentration (20 mg/L) to fry, fingerling, and juvenile rainbow trout held at 14ºC; (2) for juvenile rainbow trout (which were the most sensitive life stage tested) held at 14ºC, the margin of safety extends to at least 40 mg/L; (3) for fry and fingerlings held at 14ºC, the margin of safety may extend to at least 60 mg/L; and (4) the margins of safety for all three life stages may be even higher at colder water temperatures.

A 24-day study to evaluate the acute toxicity of chloramine-T when test fish were exposed on three consecutive days is scheduled to begin on August 21, 2000 at the NIO.

Histopathology: Study with cold water fish exposed to chloramine-T on three alternate days has been completed. Study results show that: (1) no pathologies were detected in eye, skin, liver, or kidney tissue; (2) severe pathological changes occurred in gills of (moribund) fish exposed to 60, 80, or 100 mg/L chloramine-T. These changes were not apparent in gills of fish sampled 7 and 14 days after the third chloramine-T exposure; (3) erythrophagia was present in hematopoietic tissue of fish exposed to 40, 60, 80, and 100 mg/L chloramine-T, and was still evident in fish sampled 14 d after the third chloramine-T exposure; and (4) mild hematopoietic hyperplasia was evident 7 d after the third chloramine-T exposure in fish exposed to 40, 60, 80, and 100 mg/L chloramine-T, and was also evident in fish sampled 14 d after the third chloramine-T exposure. Increasing erythrophagia and hematopoietic hyperplasia were observed with increasing chloramine-T concentration. However, NIO concluded that the proposed maximum efficacious chloramine-T treatment concentration (20 mg/L) is safe to use on juvenile rainbow trout being held at $\leq 14^{o}$C. In addition, the margin of safety extends to at least 40 mg/L, in spite of the moderate pathological changes associated with erythrophagia and hematopoietic hyperplasia. A report summarizing results from the above studies is currently being drafted.

Current Status: Target animal safety tests at UMESC with fry and fingerlings of three cool water and two warm water fish species have been completed. Additional exposures (initiated in Project Year No. 6) are in progress with walleye fingerlings to obtain histological samples required 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. Additional exposures are also in progress to test the effect of water quality on walleye fingerlings, a species sensitive to chloramine-T. The post-treatment phase of the walleye study is currently in progress and will be completed in August 2000. The results of this study will be summarized in a report.

Target animal safety tests at NIO with fry, fingerling, and juvenile life stages of rainbow trout have been completed and the results of these studies will be summarized in a report. A study to evaluate the toxicity of chloramine-T when exposed to rainbow trout on three consecutive days has been scheduled by NIO for 2000.

STUDY NUMBER 5: (NEW TITLE) APPROVAL OF FLORFENICOL AS AN ORAL DRUG TO CONTROL SUSCEPTIBLE SYSTEMIC BACTERIAL DISEASES IN FRESHWATER FISH

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.

NOTE: The IAFWA DAWG, formerly the Drug Approval Oversight Committee voted to redirect funds and efforts from florfenicol to efforts toward approvals of other IAFWA Project drugs at its meeting during the North American Fish and Wildlife Conference on March 26, 2000. The progress reported in this section reflects progress made prior to March 26, 2000.

Job No. 1: Support of INAD request 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: An analytical method for the determination of florfenicol in fish feeds, developed for Schering-Plough Animal Health by a Canadian contract laboratory, was evaluated by UMESC scientists using a Canadian salmonid feed and validated on salmonid feeds from several US manufacturers.

Initiation by NIO of work under Schering-Plough's INAD for florfenicol to control systemic infections in freshwater fish has not begun.

Current Status: UMESC scientists completed a preliminary validation of the method used to analyze florfenicol in fish feeds. Comparable results verified the study completed for Schering-Plough Animal Health on Canadian fish feed. Before UMESC chemist can analyze fish feed samples obtained from pivotal efficacy studies conducted by NIO they must be certified under a validation program coordinated by Schering-Plough Animal Health. The certification will allow UMESC chemists to analyze feed in support pivotal efficacy trials in a variety of species.

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

Progress: No activity July 1, 1999 to June 30, 2000.

Current Status: Effort on the florfenicol analytical method in salmonid tissues was redirected to similar studies with chloramine-T. Schering-Plough's analytical method in edible fish tissue is inadequate for some species of fish.

Work in this job was redirected to other IAFWA Project drugs at the direction of the IAFWA DAWG on March 26, 2000.

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

Progress: No activity July 1, 1999 to June 30, 2000.

Current Status: The National NADA Coordinator and UMESC scientists had originally planned to work with the sponsor and CVM to identify data requirements and how they will be met. Research in Job 3, programmed to have begun in IAFWA Project Year No. 6, has been stopped at the direction of the IAFWA DAWG on March 26, 2000 and reprogrammed to work on other IAFWA Project drugs.

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

Progress: The National NADA Coordinator assisted Dr. Randy MacMillan from the private sector in addressing issues on antimicrobial resistance raised by a staff of the Centers for Disease Control and Prevention (CDC). A response was sent to CDC on December 20, 1999.

Current Status: Concern for the development of microbial resistance of human pathogens will still need to be addressed for all food animal antimicrobials including florfenicol. The National NADA Coordinator will continue to work with interested groups to address this issue even though work on florfenicol was redirected by the IAFWA DAWG on March 26, 2000.

Job No. 5: Prepare an environmental assessment of the fate and effects of the release of florfenicol.

Progress: A hatchery survey was developed by UMESC scientists and distributed by the IAFWA DAWG chairman to public aquaculture facilities and by the National NADA Coordinator to private aquaculture facilities. The purpose of the survey was to identify potential use patterns of florfenicol among the aquaculture community in anticipation of drafting an amended environmental assessment.

Current Status: Results of the hatchery survey describing the anticipated use of florfenicol at public and private hatcheries were summarized and submitted to the sponsor. Fifty of the 100 hatcheries that responded to the survey indicated the potential to treat fish with florfenicol. Most respondents were interested in the treatment of columnaris and bacterial coldwater disease or furunculosis. Treatments were anticipated throughout the year; however, fewer treatments were projected during winter. The treated biomass of fish varied with culture unit size, but average treated biomass for several different culture units was between 1000 - 5000 kg with about 13 treatments being administered during a given year.

Work in this Study was redirected on March 26, 2000 at the direction of the IAFWA DAWG.

Job No. 6: (New Title): Gain approval for a compassionate INAD exemption.

Progress: The NIO prepared two florfenicol study protocols: (1) a compassionate INAD exemption protocol and (2) a pivotal field efficacy protocol. These protocols were submitted to CVM requesting an INAD number and slaughter authorization. The FWS was granted INAD #10-687, but no slaughter authorization was given by CVM. The FWS has since deactivated the INAD due to indications from the sponsor, Schering-Plough Animal Health, that they will not support a compassionate INAD use of florfenicol.

Current Status: Pivotal field efficacy studies are planned at several FWS facilities, as well as at facilities identified through the FWS INAD piggybacking program but under the sponsor's INAD. Studies will be conducted to evaluate the efficacy of florfenicol to control mortality in a variety of salmonids caused by either columnaris and coldwater diseases.

STUDY NO. 6: APPROVAL OF POTASSIUM PERMANGANATE 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 a external microbicide for fish.

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

Job No. 2: (Complete) 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 environmental assessment of the fate and effects of release of potassium permanganate treated water.

Progress: CVM is requiring an environmental assessment, not a categorical exclusion as requested, on the use of potassium permanganate in aquaculture. Carus Chemical Company is negotiating with University of Mississippi for preparation of the environmental assessment. SNARC will work with the University of Mississippi to complete and submit the EA data package.

Current Status: Negotiations are in progress between Carus Chemical Company and University of Mississippi for Carus to fund the development of an environmental assessment for potassium permanganate. Once complete, the University of Mississippi, in collaboration with SNARC will conduct an environmental assessment.

Job No. 4: Conduct studies of manganese residues in organs and tissues of cultured food fish other than channel catfish exposed to potassium permanganate at therapeutic levels.

Progress: No activity July 1, 1999 to July 30, 2000.

Current Status: Analysis of samples of rainbow trout tissue to quantify manganese residues following exposure to waterborne potassium permanganate has been completed and results have been returned to Clear Springs Food Company in Buhl, Idaho. Clear Springs Food Company will prepare the final report of the study and submit it to CVM.

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

Progress: Efficacy of potassium permanganate for preventing establishment of

Ichthyophthiriasis in channel catfish has been demonstrated by researchers at HKD-SNARC. Research is underway to determine whether potassium permanganate can eliminate established infections of *Ichthyophthiriasis* in channel catfish. Staff at UMESC have prepared a study protocol to determine the efficacy of potassium permanganate for treating saprolegniasis in channel catfish.

Current Status: Research on the efficacy of potassium permanganate to prevent *Ichthyopthiriasis* infestations in channel catfish is complete and a manuscript has been accepted for publication. A current study is underway to determine the efficacy of potassium permanganate in eliminating established ichthyopthiriasis.

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

Progress: The exposure and sample collection phases of target animal safety studies on channel catfish have been completed at HKD-SNARC. The sample analysis phase, except for histopathology, is complete. Chillers have been installed to permit work with rainbow trout and target animal safety studies on rainbow trout are scheduled.

Current Status: A report on the safety of potassium permanganate to channel catfish is currently in preparation. The rainbow trout target animal safety study has been postponed until January 2001 so that a target animal safety study on catfish using copper sulfate can be completed.

STUDY NUMBER 7: (REVISED TITLE) APPROVAL OF AQUI-S™ AS AN ANESTHETIC AND SEDATIVE FOR FISH

Objectives: Submission of efficacy, target animal safety, and residue depletion technical data required for the approval of AQUI-S™ as an anesthetic/sedative with a short or zero 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™ as an anesthetic/sedative for fish cultured at public hatcheries.

Progress: The NIO has submitted a protocol to CVM to collect supporting efficacy data for the use of AQUI-S™ as an anesthetic/sedative for fish culture at public hatcheries. The protocol has been reviewed by CVM. Initially, CVM did not issue the INAD because of current concerns expressed by the National Toxicology Program that AQUI-S™ may have some toxic properties. After CVM reviewed residue data submitted by the sponsor, the agency authorized a 21-day withdrawal period under the sponsor's INAD. NIO then submitted an amendment to CVM also requesting a 21-day withdrawal period under FWS INAD # 10-541. CVM notified NIO that a "Right of Reference" letter is needed from AQUI-S New Zealand LTD. This letter would allow CVM to access the sponsor's data when reviewing the requested amendment.

Current Status: Until certain mammalian toxicity issues are resolved for the active ingredient in AQUI-S™, CVM will not issue a full compassionate INAD for the product with a withdrawal period. This issue was discussed at a meeting between the sponsor and CVM on November 13, 1999 (see Job 4 Progress and Current Status below). CVM would consider a limited compassionate INAD with a 21-day withdrawal time if the sponsor allows CVM to review its residue chemistry data.

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

Progress: UMESC scientists discussed human food safety requirements for AQUI-S™ with the U.S. Representative of AQUI-S New Zealand.

Current Status: Studies of AQUI-S™ residues in Atlantic salmon (*Salmo salar*) will be initiated by the sponsor when the protocol is reapproved and the radiolabeled AQUI-S™ obtained. Once this research has been completed, the need to conduct additional residue chemistry studies in Atlantic salmon or to test additional fish species for total residue loads of AQUI-S™ will be evaluated.

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

Progress: A protocol (CAP-99-00100-03) has been completed to evaluate the efficacy of AQUI-S™ in a variety of cold, cool, and warm water fish. Start of the study has been delayed due to questions related to the compound's formulation.

Current Status: Because of possible changes in AQUI-S™'s formulation and additional data requirements for hydrogen peroxide on fish eggs, initiation of laboratory work with AQUI-S™ in Project Year 6 was limited to confirmation of the new analytical method. Laboratory toxicity studies will be initiated in early Project Year 7.

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

Progress: A meeting was held with the sponsor and CVM on November 18, 1999 to discuss the mammalian safety of the active ingredient of AQUI-S™. At the meeting, the sponsor asked that CVM issue a restricted INAD until the issue with the active ingredient is resolved. CVM responded by asking for additional fish residue chemistry data. The data were supplied to CVM in early December 1999.

Current Status: After CVM reviewed residue data submitted by the sponsor, the agency authorized a 21-day withdrawal period under the sponsor's INAD. The limited compassionate INAD under NIO is still pending waiting for action from the sponsor.

Job No. 5: Prepare an environmental assessment of the fate and effects of the release of AQUI-S™.

Progress: The drug sponsor submitted an environmental assessment of the use of AQUI-S™ in net pen aquaculture of Atlantic salmon to CVM on November 13, 1998.

A hatchery survey was developed by UMESC scientists and distributed by the Drug Approval Oversight Subcommittee chairman to public aquaculture facilities and by the National NADA Coordinator to private aquaculture facilities. The purpose of the survey was to identify potential use patterns of AQUI-S™ among the freshwater aquaculture community in anticipation of drafting an amended EA from the original submitted by the sponsor.

Current Status: Environmental fate and effects information has been submitted to CVM 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™ in freshwater pond and raceway applications.

STUDY NO. 8: DEVELOPMENT OF HYDROGEN PEROXIDE 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 potentially, for controlling external parasitic infestations and mortalities associated with external bacterial infections on freshwater fish.

Expected Products: (A) NADA technical sections submitted 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. © Assessments of the efficacy of hydrogen peroxide to control external parasitic infestations and external bacterial infections on fish will be submitted to the hydrogen peroxide public master file.

Change in status: Hydrogen peroxide will retain its current Low Regulatory Priority (LRP) status for the foreseeable future to control 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: Pivotal efficacy studies are completed for a label claim to control mortalities on fish eggs resulting from saprolegniasis. UMESC submitted an INAD protocol to CVM (INAD 10-023) to ensure that supporting data from public hatcheries are collected to support the efficacy label claims.

Efficacy to Fish Eggs: CVM reviewed studies submitted to document the efficacy of hydrogen peroxide to control mortality associated with saprolegniasis on the eggs of cold, cool, and warm water fish. CVM stated that the data submitted were sufficient to support a label claim that a 15 min treatment at 500 mg/L controlled mortalities associated with saprolegniasis in rainbow trout eggs and that the data were sufficient to make an all salmonid egg claim. CVM did not agree that the data submitted supported a label claim that treatments administered at 500 mg/L for 15 min were efficacious to control mortalities associated with fungal infections for cool and warm water fish eggs. In a conference call between UMESC and CVM, UMESC agreed to provide additional efficacy data for one warm water species (paddlefish) using concentrations of between 250 and 1,500 mg/L to better delineate the efficacy of hydrogen peroxide. CVM also requested that the eggs be inoculated with a known species of fungus at the initiation of the trial. UMESC initiated a pivotal efficacy study with paddlefish eggs in late Project Year 6. Paddlefish eggs were exposed to fungal zoospores *(Saprolegnia parasitica)* by placing tea balls containing hemp seeds infected with fungus in the head box of the test system. The eggs were treated daily for 15 minutes with 250, 500, 1,000, or 1,500 mg/L of hydrogen peroxide and the tea balls were remove after fungus was observed on the eggs. The eggs of both treated and control groups became severely infected with fungus and all eggs died before hatch.

A protocol was submitted to CVM on March 3, 2000 by UMESC requesting to amend an existing INAD held by UMESC (INAD 10-023) to allow public and private hatcheries to collect pivotal and supporting efficacy data to support a label claim for hydrogen peroxide to control mortalities associated with saprolegniasis on fish eggs. Pivotal and supporting efficacy trials were conducted by seven public and private facilities on channel catfish, walleye, smallmouth bass, and paddlefish. The infectious agent was identified from three hatcheries as *Saprolegnia ferax* or *Saprolegnia parasitica.* Preliminary results indicated that although hydrogen peroxide did not increase egg hatch in comparison to controls, hydrogen peroxide treatment did reduce or eliminate saprolegniasis on treated eggs.

Current Status: UMESC will summarize the data collected at participating hatcheries and submit a report to CVM in Project Year 7.

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: Two technical section submissions on efficacy were submitted on January 28, 2000 and May 1, 2000 to CVM that support the use of hydrogen peroxide to control external bacterial infections and external parasitic infestations on freshwater fish.

A study was conducted to compare the efficacies of hydrogen peroxide and chloramine-T to control mortalities associated with external flavobacter infections on cultured fish at the Rathbun Hatchery, Iowa. Walleye infected with columnaris pathogens were treated with 50, 100, or 150 mg/L of hydrogen peroxide for 30 minutes every other day on three occasions. The same lot of fish were treated with 20 and 30 mg/L of chloramine-T for 60 minutes every other day on three occasions. The results from these studies will provide a comparison on the efficacy of the two compounds to control mortalities associated with bacterial infections.

Two protocols were submitted to CVM on April 18, 2000 by UMESC requesting an amendment to an existing INAD held by UMESC (INAD 10-023) to allow public and private hatcheries to collect pivotal and supporting efficacy data to support label claims for hydrogen peroxide to control (1) mortalities associated with external bacterial infections on fish and (2) external parasite infestations on fish. Seven hatcheries have offered to conduct efficacy trials for external bacteria and eight for external parasites.

Current Status: Preliminary observations of pivotal dose titration studies indicate that hydrogen peroxide bath treatments may control mortalities associated with bacterial gill disease and control certain external parasitic infestations on freshwater fish. All laboratory work is assumed complete pending CVM review of the final reports submitted by UMESC.

The post-treatment phase of the study at the Rathbun Hatchery, Iowa is currently in progress and will be completed in August 2000. The results of this study will be summarized in a report.

To date, no supporting efficacy trials have been conducted under a compassionate INAD for Perox-Aid™ to control mortalities associated with bacterial gill disease and flavobacteriosis on fish and to control external parasitic infestations on fish.

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

Progress: CVM reviewed target animal safety studies conducted on cold, cool, and warm water fish eggs. CVM did not agree with UMESC's assessment that hydrogen peroxide administered at 1,000 mg/L for 15 min through hatch was safe for all fish species. In a conference call between UMESC and CVM, UMESC agreed to provide additional safety data on eggs for one cold and one warm water species using concentrations of between 1,000 and 2,500 mg/L to better delineate the toxicity of hydrogen peroxide. The reason for CVM's concern relates to the fact that many or most eggs from cool water and warm water fish are infected with external fungal infections before they are received at UMESC.

Target animal safety studies for fish have been completed. A report will be submitted to CVM in 2000.

Current Status

Target Animal Safety Studies with Eggs: Additional target animal safety studies were completed with rainbow trout and paddlefish eggs at 12°C and 17°C, respectively. No additional studies are currently planned. Green eggs of both species were received at UMESC within 48 h of fertilization and placed into miniature egg jars for treatment. Fifteen minute hydrogen peroxide treatments of 0, 1,000, 1,500, 2,000, or 2,500 mg/L were administered daily until hatch was complete. Remaining live and dead eggs as well as live and dead fry were enumerated after hatch was complete. Study data is currently being summarized and a report will be prepared for submission to CVM in Project Year 7.

Target Animal Safety Studies with Fish: A report documenting the acute and sub-acute toxicity

of hydrogen peroxide exposure to fish is in review by the UMESC Quality Assurance Unit and will be submitted to CVM in 2000 to fulfill the target animal safety technical section requirements for all fish species.

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

Progress: An environmental assessment for hydrogen peroxide use in intensive aquaculture operations was prepared by UMESC scientists and submitted to CVM on March 14, 2000. The assessment includes descriptions of the magnitude and breadth of hydrogen peroxide treatments of (1) fish eggs to control saprolegniasis in incubators and (2) fish to control saprolegniasis, external bacterial infections, and external parasitic infestations in hatchery tanks and raceways. An assessment of the magnitude of effects of hatchery effluent entering fresh or brackish water ecosystems was made. The EA does not address hydrogen peroxide use in pond aquaculture because it is expected that this use will not be economically viable. Data for the environmental assessment was developed from a survey of public (state) and private hatcheries, and the public literature.

To date, 100 hatcheries, representing 23 different states, have responded to the survey, which is still posted on the UMESC website. Information from 92 hatcheries was summarized in the submission. Most hatcheries responding to the survey discharge their hatchery effluents into rivers or streams (48/92) with the remainder discharging into standing bodies of water (e.g., lakes or backwaters). The median daily water use for each responding hatchery was 3.1 million gal per day.

Information from the survey was incorporated into the EA for hydrogen peroxide and played a key role in developing realistic exposure/risk models of the safety of hydrogen peroxide in receiving waters. The knowledge developed, while preparing models for hydrogen peroxide dispersion in rivers and streams, will be easily transferred to other chemicals such as AQUI-S™, copper sulfate, chloramine-T, florfenicol, and potassium permanganate to support development of their EA submissions.

Estimated environmental concentrations (EEC) of hydrogen peroxide were computed from information provided in the survey for the EA. Of the hatcheries responding to the survey, 36 reported use or intended use of hydrogen peroxide on eggs and 31 reported use or intended use of hydrogen peroxide on fish. Based on the information reported by the hatcheries that indicated current or planned use of hydrogen peroxide, UMESC estimated EECs in the receiving water bodies that range from 0.09 mg/L 45 min (after a typical hatchery treatment) to 0.01 mg/L 96 h after a typical hatchery treatment with hydrogen peroxide. Hazard quotient and level of concern analyses suggest no increased risk associated with release into freshwater or saltwater. Assessment of the EECs by UMESC scientists suggest that chemical mitigation of hydrogen peroxide treatments will likely not be required.

Current Status: The environmental assessment (EA) for hydrogen peroxide was submitted to CVM by UMESC staff on March 14, 2000 and is under review by CVM.

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

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. Additional work would need to be undertaken beyond the fifth year of the IAFWA Project to complete crop grouping for the model oral drug.

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

Progress: Details for the performance of the method to determine florfenicol in fish plasma are nearly complete, however progress has been delayed because a key individual in the research was required to draft submissions for environmental assessments of hydrogen peroxide and chloramine-T.

Current Status: Assessment of the analytical method to determine florfenicol in fish plasma has been inactive during the completion of studies on other IAFWA Project drugs. Pharmacokinetic studies can proceed once the method in plasma is validated in a fish species.

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

Progress: No activity July 1, 1999 to June 30, 2000.

Current Status: Studies in Job No. 2 will be initiated after completion of pharmacokinetic studies for florfenicol in Job No. 1.

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

Progress: The crop grouping concept was tested with the water borne drug benzocaine in four diverse fish species important to public aquaculture. The comparative metabolism, tissue distribution, and elimination of benzocaine was studied in rainbow trout, channel catfish, yellow perch, and lake sturgeon by (1) direct analytical chemistry studies using radiolabeled benzocaine, (2) classical compartmental pharmacokinetic evaluations of benzocaine in the four species, and (3) developing and validating physiologically based pharmacokinetic models of the drug in channel catfish and rainbow trout.

While interspecies and temperature-induced differences in the pharmacokinetics were observed among the four species of fish tested, the differences were relatively small such that it was not possible to identify a Crop Grouping among the diverse groups. All four species were studied at the common temperature of 16°C and half lives were similar in catfish and trout (6-8 hr.), but were about half those observed in perch and sturgeon. The half lives were temperature sensitive in catfish (16, 21 and 26°C) and trout (7, 12 and 16°C), but the sensitivity was not great and worked in opposite directions in the two species. With increasing temperature, half life of elimination increased in the warm water catfish and decreased in cold water trout. Plasma protein binding was similar in the four species and was insensitive to change in temperature.

The metabolite profiles of benzocaine were common to all four species however the intrinsic capacity in yellow perch to metabolize benzocaine was only about one-quarter that of the other species.

The PBPK model for benzocaine that was developed for channel catfish identified four parameters that most strongly influenced the persistence of benzocaine in plasma and muscle tissue. These parameters were the (1) free fraction of benzocaine in plasma, (2) fat/blood partition coefficient of benzocaine, (3) fraction of the fish body that was fat, and (4) blood flow to the fat tissue. Environmentally induced (i.e. temperature) and interspecies differences exaggerated the effect that these parameters had on plasma and muscle benzocaine concentrations. Benzocaine persistence in plasma and muscle was insensitive to 24 other PBPK model parameters.

In contrast to channel catfish, the PBPK model for rainbow trout indicated that the persistence of benzocaine in plasma and muscle was influenced by five different parameters. They were: 1) the liver volume fraction of the whole body; 2) the kidney volume fraction of the whole body; 3) the richly perfused tissue volume fraction of the whole body; 4) the benzocaine perfusion distance across the gill; 5) the concentration of saturable benzocaine plasma protein binding sites. The model was not sensitive to 39 other parameters.

Monte Carlo analysis of the PBPK model for rainbow trout indicated that predicted mean concentrations of benzocaine in the plasma and muscle from the model were in good agreement with observed concentrations over the time course of the experiments suggesting that the model was performing adequately.

Current Status: The experimental phase of the Crop Grouping research being conducted at Ohio State University is complete and a final report is in preparation. Preliminary results indicate that there are more similarities than differences in the manner in which diverse groups of fish handle benzocaine. Evaluation of the PBPK model by Monte Carlo analysis has been completed for the rainbow trout. Evaluation of the PBPK model for channel catfish by Monte Carlo analysis is currently being undertaken. The expected results are that the analysis of the data should give reliable predictions of the residue concentration variability of benzocaine and its metabolites in the tissue of exposed fish. Validation of the combined PBPK/Monte Carlo model analyses will then be possible when predictions of residue concentration are compared to actual concentrations determined for exposed fish. Once validated, it should be possible to use the model to predict residue concentrations in a variety of fish species. As a word of caution, the benzocaine PBPK

model in channel catfish is particularly sensitive to the chemical's physico-chemical properties. It is likely that this also will be true of most drugs, so that the physico-chemical properties of a drug will likely influence greatly the pharmacokinetic properties of the drug in most fish or fish groups. Thus, future research in this area will likely need to not only key on diverse fish species, but also on drugs with diverse physico-chemical properties.

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

Progress: The comparative metabolism, tissue distribution, and elimination of benzocaine was studied in rainbow trout, channel catfish, yellow perch, and lake sturgeon at a common temperature of 16 °C by (1) direct analytical chemistry studies using radiolabeled benzocaine and (2) classical compartmental pharmacokinetic evaluations of benzocaine in the four species. While interspecies differences in the pharmacokinetics were observed among the four species of fish tested at the common temperature, the differences were relatively small such that it was not possible to identify a Crop Grouping among the diverse groups.

The benzocaine plasma concentration-time profile declined in a biexponential fashion after intra arterial infusion administration in all species. The pharmacokinetics was best described as a two compartment model. When all four species were studied at the common temperature of 16 °C, the terminal elimination half lives were similar in catfish (7.8 hr) and trout (5.8 hr), but were about half those observed in yellow perch (12.3 hr) and lake sturgeon (16.5 hr). The qualitative metabolite profiles of benzocaine in the various tissues were common to all four species indicating that benzocaine metabolism followed a common metabolic pathway. The intrinsic capacity to metabolize benzocaine was different among the species as suggested by the relative concentration profiles of the metabolites in the terminal elimination phase. Benzocaine generally occurred in the plasma in the highest concentration when compared to its metabolites in channel catfish, yellow perch and lake sturgeon. Acetylated para aminobenzoic acid was greatest in the rainbow trout followed by benzocaine. However, unlike rainbow trout, channel catfish, and yellow perch whose two lowest relative rank order metabolite concentrations were acetylated benzocaine and para aminobenzoic acid, the two lowest plasma metabolites in lake sturgeon were acetylated para aminobenzoic acid and acetylated benzocaine. The differences are likely caused by the rapid intrinsic rate of metabolism of benzocaine in the liver of lake sturgeon. The percent of plasma protein binding was common in all species however intrinsic rates of clearance in the liver was greatest in the lake sturgeon (1.91 ml/hr/g) and rainbow trout (1.54 ml/h/g) and lowest in the channel catfish (0.92 ml/h/g) and yellow perch (0.27 ml/h/g).

Current Status: Studies of crop grouping for the water borne drug benzocaine have been completed in four species and the results have been analyzed. Arrangements have been made to present the results to CVM in the form of a seminar/meeting on August 30, 2000 at the Office of Research. After the meeting, a terminal completion report for this phase of the crop grouping research will be prepared and formally submitted to CVM.

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/Status

GENERAL: In its meeting on September 8, 1999, the Minor Use/Minor Species (MUMS) Coalition asked CVM a series of questions and supplied a list of provisions it supports in general. CVM was very supportive of all the provisions and answered all the questions raised by the MUMS Coalition. On September 9, 1999 the MUMS Coalition met to discuss the development of legislation to be called the Minor Animal Species Health and Welfare Act of 2000 and the strategies for getting the legislation through Congress. The MUMS Coalition again met on December 1, 1999 in Chicago, Illinois to review the draft legislation. The legislation was refined over the next several months and introduced into the House of Representatives on June 27, 2000 and plans are underway to introduce the Bill into the U.S. Senate in early September 2000. Efforts are being made to add co-sponsors in both Congressional bodies.

The National NADA Coordinator organized, chaired, and gave the keynote address at a session on international harmonization of antibacterial approvals and sensitivity testing at the European Association of Fish Pathologists 9th International Conference, September 19-24, 1999 in Rhodes, Greece.

The National NADA Coordinator contributed to a white paper that addresses critics that suggest that use of antibacterials in aquaculture is a potential hazard to humans because the use can lead to antimicrobial resistance in humans. She also reviewed a draft of the white paper that was in response to allegations made by an official at the Centers for Disease Control and Prevention (CDC). These activities occurred in September through December 1999. The response was sent to CDC on December 20, 1999.

The Joint Subcommittee on Aquaculture (JSA) formed the Aquaculture Effluents Task Force 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 EPA. The task force has had several meetings in 2000 to set the course of action. The National Aquaculture NADA Coordinator is a member of Subgroup for Drugs and Chemicals and drafted a white paper for submission to EPA in June 2000. The white paper was reviewed by the CVM Environmental staff and sent to EPA on August 24, 2000.

In May 1999, Dr. Joan Gotthardt replaced Dr. Tom Bell at CVM for reviewing aquaculture drug

submissions. On September 27, 1999, CVM hired Donald Prater, a second technical reviewer for efficacy and target animal safety data submissions for aquaculture drugs. Dr. Gotthardt became the leader of the newly formed Aquaculture Drugs Team (HFV-131) at CVM in the summer of 2000. The backlog in efficacy and target animal safety submissions have been eliminated as a result of the efforts of the Aquaculture Drugs Team.

The National NADA Coordinator was taped on November 17, 1999 for a video on the benefits and need for INAD participation.

A website established for the National NADA Coordinator on April 12, 1999 at http://ag.ansc.purdue.edu/aquanic/jsa/aquadrugs/index.htm was updated in August 1999 and in May 2000.

The National NADA Coordinator received the FDA Commissioner's Special Citation Award at a ceremony in Rockville, Maryland on June 9, 2000 for outstanding leadership, teamwork, and sustained efforts as the National NADA Coordinator.

Specific Drugs

AQUI-S™ — Status: Sponsor (AQUI-S New Zealand LTD) proceeding with worldwide drug approval. *BOTTOM LINE:* All submissions should be completed by 2002 for zero or low withdrawal time anesthetic for salmonids and for all fish (?)

Progress on Technical Sections:

- ***Product Chemistry*** — Accepted elsewhere; no current activity for U.S.
- ***Mammalian Safety*** — The sponsor (AQUI-S New Zealand LTD) 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 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. for original active ingredient based on their assessment of scientific data that the active ingredient is not a carcinogen.
- ***Environmental Safety*** — The sponsor submitted a summary to CVM and has completed an environmental biodegradation study in freshwater.
- ***Residue Chemistry*** — On July 7, 1999, the sponsor signed a CRADA with UMESC (in progress)
- ***Target Animal Safety*** — Preliminary toxicity studies have been completed at UMESC on a variety of fish species. Pivotal target animal safety studies are in progress at UMESC. The sponsor is ready to submit target animal safety and efficacy studies on salmonids completed in Canada to CVM.
- ***Efficacy*** — Preliminary efficacy studies were completed at UMESC on a variety of fish species. Pivotal efficacy studies are in progress at UMESC. The sponsor is ready to submit efficacy studies on salmonids completed in Canada to CVM. The sponsor submitted existing residue depletion rates to CVM in December 1999 that gained a 21-day

experimental withdrawal time. An INAD number was established for FWS to collect supporting efficacy data at limited sites but experimental withdrawal time and slaughter authorization is pending CVM's access to sponsor's data via a "Right of Reference" letter.

The IAFWA Drug Approval Working Group on AQUI-S™ decided on March 26, 2000 to continue to support current research on an active ingredient in AQUI-S™ 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 NTP study, (4) similar results on a related active ingredient, eugenol, and (5) similar toxicological studies showed no adverse effect.

Benzocaine — Status: Major effort by IAFWA Project for NADA approval terminated because of decision by IAFWA Project stakeholders to select AQUI-S™ as the candidate anesthetic in the U.S. public aquaculture sector; no known drug approval activities underway.

Chloramine-T (external antibacterial) — Status: Sponsor (Axcentive; 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 on salmonids and for control of mortalities associated with external flavobacteriosis on cool water fish.

The National NADA Coordinator met with Akzo Nobel Chemicals, Inc. on November 29, 1999 in La Crosse, Wisconsin to discuss the remaining data requirements for chloramine-T and strategies to met those data requirements.

On April 25, 2000, the National NADA Coordinator was informed that Akzo had sold its chloramine-T product to two of its employees and the new company's name is Axcentive. All contacts, agreements, and timetables will remain the same.

Progress on Technical Sections:

- ***Product Chemistry*** — Sponsor, Axcentive is committed to developing the product chemistry technical section
- ***Mammalian Safety*** — Axcentive completed genotoxicity studies on p-TSA, the marker residue of concern for chloramine-T. The genotoxicity studies have not yet been submitted studies to CVM.
- ***Environmental Safety*** — Axcentive is close to signing an amended CRADA with UMESC. Axcentive has agreed to send their confidential environmental data to UMESC for development of the environmental assessment after the amended CRADA is in place. A model was developed by UMESC to estimate discharged environmental concentrations of chloramine-T based on UMESC's hatchery survey and a point source dilution model from the U.S. Geological Survey. The environmental assessment is in progress at UMESC.
- ***Residue Chemistry*** — Some residue chemistry data developed by UMESC has been accepted by CVM. Complete validation of proposed regulatory method for marker residue

and marker residue depletion studies are in progress.

- ***Target Animal Safety*** — Target animal safety studies will soon be completed on all fish at the NIO and UMESC and technical sections will then be submitted to CVM.
- ***Efficacy*** — Efficacy data requirements are met for the control of bacterial gill disease on salmonids reared in freshwater at 12 to 20 mg/L; pivotal efficacy data from Pennsylvania was accepted for prevention of bacterial gill disease in tiger muskellunge at 20 mg/L; however, CVM is not expected to approve this use when the environmental assessment is reviewed.

  Pivotal and supporting efficacy data are needed to support a label claim for control of external flavobacteriosis on cool and warm water 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 flavobacteriosis.

Clove oil — Status: Oil of cloves (eugenol) is considered Generally Recognized as Safe when used as a direct food additive (21CFR184.1257); however, to use eugenol as an anesthetic on fish, it must be approved by CVM for that purpose. *BOTTOM LINE:* A sponsor is required to proceed toward approval and no sponsor has come forward; no known drug approval activities underway.

Copper Sulfate (external microbicide) — Status: Sponsor has an acceptable product chemistry technical section. *BOTTOM LINE:* All submissions should be completed in 2000 for control *Ichthyopthirius* on catfish. The claims for control of *Ichthyopthirius* on all fish and other external microbes on all fish would be based on efficacy and target animal safety studies that would be completed in 2002 if stakeholders are interested.

The revised Ecological Risk Assessment (ERA) for the use of copper sulfate to control certain waterborne fish diseases was submitted by HKD-SNARC to CVM on June 29, 1999. CVM requested additional references that were supplied in April 2000 by HKD-SNARC.

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 is in the process on performing such a study on channel catfish.

The National NADA Coordinator prepared draft letters on July 14, 1999 that were submitted by Phelps Dodge Refining Corporation to CVM on July 15, 1999 concerning a request for exclusivity and for the writing of the human food safety portion of the Freedom of Information (FOI) summary for copper sulfate. CVM did not grant exclusivity but did prepare the FOI summary on March 3, 2000.

Progress on Technical Sections:

- ***Product Chemistry*** — Accepted by CVM from the sponsor, Phelps Dodge Refining Corporation

- ***Mammalian Safety*** — Accepted by CVM; FOI written on March 3, 2000
- ***Environmental Safety*** — The revised environmental safety technical section for all fish is in review at CVM. References related to the environmental safety were submitted by HKD-SNARC to CVM at the agency's request in April 2000.
- ***Residue Chemistry*** — Accepted by CVM; FOI written on March 3, 2000
- ***Target Animal Safety*** — Additional target animal safety studies are required for an all fish label claim. A study is in progress on channel catfish.
- ***Efficacy*** — Accepted by CVM for control of *Ichthyopthirius* on all fish

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

  Efforts are underway by HKD-SNARC to conduct pivotal efficacy studies to control fungi on fish eggs. Needed are (1) pivotal efficacy studies to control fungi on all fish, (2) control external flavobacteriosis on all fish, and (3) control external parasites (except *Ichthyopthirius)* on all fish

  FOIs for efficacy and target animal safety are underway at HKD-SNARC.

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

Florfenicol (oral antibacterial) — Status: Sponsor recently allowed the development of florfenicol for approval in U.S.; approved in Canada in August 1997 to control furunculosis in Atlantic salmon. *BOTTOM LINE:* Sponsor will continue to develop data for aquaculture approval but IAFWA Project efforts on florfenicol have been redirected at this time by the IAFWA DAWG to other IAFWA Project drugs.

A meeting was held on July 20-21, 1999 with Schering-Plough Animal Health, FWS, UMESC, and the National NADA Coordinator to discuss the details in initiating the efficacy studies through a FWS-INAD and in conducting the residue chemistry studies.

Discussions on the development of florfenicol were held with CVM at the FWS-INAD Coordination meeting in Bozeman, Montana on August 4-5, 1999.

Schering-Plough Animal Health submitted a data package to CVM for florfenicol to control furunculosis in salmonids on September 30, 1999.

Schering-Plough Animal Health reviewed the quality assurance and Good Laboratory Practices procedures at UMESC on October 5-6, 1999.

Schering-Plough Animal Health recently gained an approval in the United Kingdom for the use of

florfenicol to control furunculosis in Atlantic salmon in marine net pens.

Recent commitment of financial support from North Central Regional Aquaculture Center was made to the Iowa Department of Natural Resources for pivotal efficacy studies on percids for control of systemic flavobacteriosis.

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.

The IAFWA DAWG decided at on March 26, 2000 to redirect efforts on the only new oral antibacterial drug available to aquaculture to other IAFWA Project drugs because the development will take until at least 2002 or perhaps beyond to obtain data for all fish and all potential label claims and to resolve the antimicrobial resistance issue. DAWG, however, agreed to support florfenicol under a new funding initiative under development by IAFWA and FWS. Crop grouping research with florfenicol is still to go forward as planned.

Formalin (external microbicide) — Status: Supplemental NADA approved on June 18, 1998 for control of certain fungi on the eggs of all fin fish and certain external protozoa and monogenetic trematodes on all fin fish. *BOTTOM LINE:* All submissions should be completed in 2000 for control of mortalities associated with fungal infections on salmonids and by 2002 for control of mortalities associated with fungal infections on <u>all</u> fish.

Progress on Technical Sections:

- ***Product Chemistry*** — Accepted by CVM
- ***Mammalian Safety*** — Accepted by CVM
- ***Environmental Safety*** — Accepted by CVM
- **Residue Chemistry** — Accepted by CVM
- **Target Animal Safety --** Accepted by CVM
- ***Efficacy*** — Fungal disease model developed for efficacy studies by UMESC

  CVM informally accepted supporting efficacy for control of fungi on salmonids from FWS and UMESC efforts. Plans are underway by CVM to perform pivotal efficacy studies for control of fungi on salmonids. Supporting efficacy data are needed for control of fungi on cool and warm water fish.

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 be completed in 2000 for control of mortality from saprolegniasis on all fish eggs, in 2001 for control of mortality from saprolegniasis on all fish , and in 2002 for control of mortality from external flavobacteriosis and bacterial gill disease on all fish and to control parasitic infestations on all fish.

The sponsor (Eka Chemicals Inc.) has completed the negotiations with Syndel International Inc. to do the marketing of hydrogen peroxide (Perox-Aid™) for fisheries use in Canada.

The sponsor (Eka Chemicals Inc.) submitted a product chemistry technical section on July 12, 1999 and a request to CVM to write the FOI summary on November 10, 1999. CVM responded to both submissions on January 7, 2000 and March 22, 2000, respectively.

A series of meetings were held in July, August, October, and November 1999 at UMESC with the National NADA Coordinator to discuss the development of the remaining data requirements, especially environmental safety, to complete the submission of all the technical sections needed for approval. Several conference calls were held with the sponsor as well during this time period.

Progress on Technical Sections:

- ***Product Chemistry*** — Sponsor, Eka Chemicals, Inc., submitted product chemistry technical section on July 12, 1999; additional data are required that the sponsor is committed to provide.
- ***Mammalian Safety*** — Accepted by CVM. The FOI summary was written by CVM on March 22, 2000.
- ***Environmental Safety*** — 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 wrote an environmental assessment to support an all fish label claim and submitted it to CVM on March 14, 2000
- ***Residue Chemistry*** — Accepted by CVM. The FOI summary was written by CVM on March 22, 2000.
- ***Target Animal Safety*** — Target animal safety technical section on all fish eggs was submitted by UMESC to CVM. The safety data was accepted for a dose range of 500–1,000 mg/L for northern pike, lake trout, and common carp. A target animal safety study on rainbow trout eggs and eggs of most sensitive warm water species will be completed in 2000. The target animal safety technical section on all fish will soon be submitted to CVM by UMESC.
- ***Efficacy*** — A fungal disease model was developed for efficacy studies with fish by UMESC.

  An efficacy technical section supporting the control of fungi on all fish eggs was submitted by UMESC to CVM; CVM accepted efficacy data for control of mortality from saprolegniasis on salmonid eggs. However, more data were needed for an all fish egg claim: (1) pivotal efficacy studies in progress by UMESC to support a claim for control of mortality from saprolegniasis on cool and warm water fish eggs and (2) supporting efficacy studies still needed to support a claim for control of mortality from saprolegniasis on cool and warm water fish eggs.

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

Pivotal efficacy studies on control of mortality from external flavobacteriosis and control of external parasites on salmonids were submitted by UMESC to CVM on January 28, 2000 and May 1, 2000, respectively; however, the following efficacy data are still needed: (1) supporting efficacy data for control of mortality from external flavobacteriosis and control of external parasites on salmonids and (2) both pivotal and supporting data for control of mortality from external flavobacteriosis and for control of external parasites on cool and warm water fish.

Protocols for supporting efficacy studies under an INAD at UMESC were submitted to CVM for review for (1) control of mortality from saprolegniasis on cool and warm water fish eggs and all fish, (2) control of mortality from external flavobacteriosis on all fish, and (3) control of external parasites on all fish.

When target animal safety technical section on salmonid eggs and the environmental assessment are accepted and the FOIs completed for efficacy and target animal safety, the data requirements will be completed for the control of mortality from saprolegniasis on salmonid eggs.

Oxytetracycline (OTC, 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 should be completed in 2000 for otolith marking on all fish, for control of *Aeromonas sp.* in cool water fish by 2002, for systemic coldwater disease in salmonids by 2002, and for control of mortalities associated with systemic columnaris disease in salmonids and walleye by 2002.

The sponsor, Pfizer Inc., is in the process of selling its oxytetracycline products to a company that has expressed an interest in aquaculture; the company has stopped production of its product for aquaculture in Canada.

FDA liaison to NRSP-7 completed a Public Master File for a supplemental NADA approval as a marking agent for all fish by immersion and submitted it to CVM in November 1999. All the technical section submissions for OTC for this use should be complete.

Progress on Technical Sections:
All technical sections except for portions pivotal and supporting efficacy are complete for new label claims to extend to other fish species and to expand its use to other diseases not on the current label (see below)

- ***Product Chemistry*** — Accepted by CVM
- ***Mammalian Safety*** — Accepted by CVM
- ***Environmental Safety*** — Accepted by CVM
- ***Residue Chemistry*** — CVM had previously approved certain label claims for OTC for cold water species above 9°C and warm water species above 16°C. Residue chemistry studies were submitted by UMESC to CVM for use of OTC below the label claim limit of 9°C. The HPLC method developed by UMESC was accepted to detect product in feed and fish tissue. The bridging study by UMESC from the HPLC method to the microbial assay

method was accepted. Residue depletion studies were completed in two cool water species by UMESC.

- ***Target Animal Safety*** — Accepted by CVM for catfish, salmonids, and lobsters. Studies may be needed on cool water fish.
- ***Efficacy*** — The efficacy technical section developed by UMESC from a data call-in was accepted as supporting data for (1) control of *Aeromonas sp.* in cool water species and (2) systemic flavobacteriosis in salmonids. Pivotal efficacy data are still needed for control of *Aeromonas sp.* in cool water species and systemic flavobacteriosis in salmonids. Both pivotal and supporting efficacy data are still needed for control of systemic flavobacteriosis in cool and warm water fish.

  Several pivotal efficacy studies were completed by FWS to control systemic flavobacteriosis in salmonids; an efficacy technical section will soon be submitted to CVM; however, more studies are still needed.

  There was a recent commitment of financial support from North Central Regional Aquaculture Center to Iowa for pivotal efficacy studies in percids for control of columnaris disease.

Potassium Permanganate (external microbicide) — Status: Sponsor recently submitted a product chemistry technical section and a request for categorical exclusion for environmental safety. *BOTTOM LINE:* All submissions should be completed by 2002 for control of *Ichthyopthirius* on all fish, for control of mortality from external fungal infections on all fish (?) and for control of mortality from external flavobacteriosis and bacterial gill disease and control of parasitic infestations on all fish (?).

Progress on Technical Sections:

- ***Product Chemistry*** —The sponsor, Carus Chemical Company, submitted product chemistry technical section for all fish to CVM on December 8, 1998; additional data are still needed
- ***Mammalian Safety*** — Data requirements are dependent upon results of residue chemistry review by CVM
- ***Environmental Safety*** — The sponsor submitted a request for a categorical exclusion from an environmental assessment for all fish to CVM on February 23, 1998; CVM is requiring an environmental assessment. The sponsor is working on developing a contract for an environmental assessment but the company needs further information on data requirements from CVM.
- ***Residue Chemistry*** — The residue chemistry technical section for all fish was submitted by HKD-SNARC to CVM and an informal response from CVM indicated that there is no increase in manganese content as a result of exposure.
- ***Target Animal Safety*** — 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 in early 2001.
- ***Efficacy*** — HKD-SNARC completed pivotal efficacy studies that demonstrate efficacy to

prevent *Ichthyopthirius* on channel catfish and tilapia. HKD-SNARC is initiating a pivotal efficacy study for control of *Ichthyopthirius* on channel catfish and a scaled species.

Staff at UMESC have prepared a study protocol to determine the efficacy of potassium permanganate for control of mortality from saprolegniasis on channel catfish.

Supporting efficacy data are considered to be sufficient by HKD-SNARC for (1) control of mortality from saprolegniasis on fish and (2) control of mortality from external flavobacteriosis, and (3) control of external parasites (including *Ichthyopthirius)* on all fish. Needed are supporting efficacy studies for control of mortality 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. *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. On March 26, 2000, the IAFWA DAWG decided to redirect efforts on the only new oral antibacterial drug available to aquaculture to other IAFWA Project drugs because the development of data on florfenicol will take at least until 2002 and perhaps beyond to obtain data for all fish and all potential label claims and to resolve the antimicrobial resistance issue. The IAFWA DAWG did agreed to support florfenicol under a new funding initiative under development by IAFWA and FWS; however, crop grouping research with florfenicol will continue under the current IAFWA Project.

Crop grouping--Status: Studies of crop grouping for the water borne drug benzocaine have been completed in four species and the results have been analyzed. Arrangements have been made to present the results to CVM in the form of a seminar/meeting on August 30, 2000 at CVM's Office of Research. After the meeting, a terminal completion report for this phase of the crop grouping research will be prepared and formally submitted to CVM.

Classical compartmental pharmacokinetic models are being developed in five phylogenetically diverse species. UMESC is using physiologically based pharmacokinetic models developed in channel catfish and rainbow trout to support work on florfenicol. Work on florfenicol in the crop grouping work plan is constrained by the inability to obtain supplies of radiolabeled florfenicol. Once radiolabeled florfenicol is obtained, the crop grouping work will continue.

THE BOTTOM LINE: Acceptance of the crop grouping concept by CVM will reduce residue chemistry data requirements and costs of approvals for all aquaculture drugs.

Current Status: All eight drugs being developed for aquaculture use under the IAFWA Project had partial or complete NADA technical sections submitted to support some form of NADA approval by end of Year 6 (June 30, 2000); however, the DAWG voted on March 26, 2000 to redirect efforts from florfenicol to other IAFWA Project drugs.

Job No. 2: Coordinate the administration of contracts.

Progress: Two existing contracts (Cooperative Agreement 14-16-0009-1562; Research Work Orders No. 20 and 25) with the Ohio State University to support the Crop Grouping concept were completed and a draft completion report was submitted to UMESC on January 12, 2000.

In July 1999 a CRADA was established between USGS and the sponsor for AQUI-S™. The CRADA will allow UMESC to work collaboratively with the sponsor to conduct required total residue depletion studies for the drug in Atlantic salmon in anticipation of submitting the human food safety technical section for an initial approval.

In June 2000, an interagency agreement was approved to fund a portion ($38,675) of the salary of the National Coordinator for Aquaculture New Animal Drug Applications for Project Year 7.

Current Status: In August 1999, the sponsor of chloramine-T (Akzo Nobel Chemicals Inc. now Axcentive) began the process to complete an amended CRADA with UMESC that will allow UMESC scientists access to confidential company environmental fate and effect data that can be used by the scientists to develop a required environmental assessment that will be submitted by Axcentive to protect their proprietary data. The amended CRADA with Akzo-Nobel Chemicals was held up when rights to chloramine-T were purchased from Akzo-Nobel Chemicals by Axcentive. A revised amended CRADA is being pursued by the new company.

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

Progress: Products (NADA submissions, study protocols, publications, special reports, and presentations) that are a part of the IAFWA Project are reported from July 1, 1999 to June 30, 2000 in Appendix I. These products will be placed in a cumulative appendix.

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 the FWS in Bozeman, MT on August 4-5, 1999. Discussions centered particularly on the status of chloramine-T, AQUI-S™, and florfenicol and the general progress of the IAFWA Project.

The IAFWA DAWG formed in 1997 to achieve its goal of obtaining drug approvals for U.S. public aquaculture held a meeting in Killington, Vermont on September 16-17, 1999 to discuss the progress being made on the IAFWA Project drugs and to support the extension of the IAFWA Project until at least 2002. The group also met on March 26, 2000 in Chicago, Illinois to get an update on the status of each drug and decide whether to continue with AQUI-S™ (yes) and florfenicol (no, but will support other funding possibilities later in 2000). See both drugs for details.

Additional efficacy studies for IAFWA Project Drugs are needed; thus, several initiatives were put forth in 2000 to add investigators. There is need for additional pivotal and/or supporting efficacy data for at least one label claim for all eight IAFWA Project drugs. The National Aquaculture NADA Coordinator wrote an article for Fish Health Section Newsletter and the National Aquaculture Association Newsletter requesting studies from the members to gain both pivotal and supporting data for IAFWA Project drugs.

Current Status: Appropriate progress reports have been and will continue to be presented to the IAFWA Project participants and stake holders. Continuing efforts will be made to inform the entire aquaculture community of the progress being made on IAFWA Project drugs and the crop grouping research.

The federal portion of the IAFWA Project has been secured until September 30, 2002 and efforts are being made to add to the 32 states who currently support the extension.

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

Progress: From July 1, 1999 to June 30, 2000, IAFWA Project personnel, sponsors, and NRSP-7 Liaison submitted, or were involved in development of 18 known packages or major requests to CVM. Some submissions are undisclosable.

AQUI-S™, Submissions.

Bell, D. 1999. Residue depletion data on AQUI-S™. Report submitted to Center for Veterinary (FDA) by AQUI-S New Zealand LTD. December 1999.

AQUI-S™, Responses.

In April 2000, CVM granted a 21-day withdrawal period to the sponsor based on the residue depletion data submitted in December 1999.

Chloramine-T, Submissions.

Bowker, J. D. 1997. Amended Report: Efficacy of chloramine-T to control mortality in apache trout caused by bacterial gill disease associated with flavobacters. Report submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA) January 10, 2000.

Bowker, J. D. 1997. Amended Report: Efficacy of chloramine-T to control mortality caused by bacterial gill disease associated with flavobacters in fall chum salmon fingerling. Report submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). January 10, 2000.

Bowker, J. D. 1997. Amended Report: Efficacy of chloramine-T to control mortality caused by bacterial gill disease associated with flavobacters in rainbow trout fingerling. Report submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). January

10, 2000.

Schreier, T. M., V. K. Dawson, and W. H. Gingerich. 1998. Amended Summary Report: Efficacy of chloramine-T treatments to control mortalities associated with bacterial gill disease on fish. Amended summary report submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). January 10, 2000. 416 pp.

Chloramine-T, CVM Responses. On July 11, 2000, CVM accepted the efficacy technical section as complete for chloramine-T to control bacterial gill disease on freshwater-reared salmonids at 12-20 mg/L for three times on consecutive or alternate days. CVM also accepted a submission from a previous year on the results of pivotal efficacy studies with chloramine-T conducted at Benner Springs Hatchery, Pennsylvania that indicated that intermittent treatments with 20 mg/L were effective in preventing mortalities in hybrid muskellunge. CVM also asked for additional data to support the proposed label claim that 5 mg/L of chloramine-T as also effective in preventing the disease.

Copper Sulfate Submissions

Griffin, B.R. and D.L. Strauss. 2000. Supplement to animal safety data for therapeutic use of copper sulfate on food fish. 13 pp. and appendices A-K. Supplement submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). January 10, 2000.

Weddle, M. 1999. Request to CVM to write the human food safety section of the FOI summary for copper sulfate. Request submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). July 15, 1999 by Phelps Dodge Refining Corporation.

Cooper Sulfate, CVM Responses. A critique of all submissions on target animal safety was informally provided by CVM in July 2000 that suggested a need for additional target animal safety studies that concentrated on histopathology.

On March 3, 2000, CVM provided a FOI summary for the human food safety of copper sulfate indicating that “All of the data show that copper does not accumulate in the edible tissue of fin fish as a result of being exposed to copper sulfate. Therefore, a tolerance, regulatory method, and withdrawal time are not needed for fin fish treated with copper sulfate”.

Florfenicol Submission (sponsor submission)

Bova, A. 1999. Control of furunculosis in salmonids. Five technical sections submitted to CVM by Schering-Plough Animal Health, Inc. Volumes submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). September 30, 1999.

Florfenicol, CVM Response. Not known. Responses to sponsors are undisclosable unless the sponsor provides information to the IAFWA Project for release to the public.

Hydrogen Peroxide submissions.

Howe, G.E., M.P. Gaikowski, L.J. Schmidt, and J.J. Rach. 2000. Environmental assessment for the proposed use of hydrogen peroxide in aquaculture for treating external fungal, bacterial, and parasitic diseases of cultured fish. Report submitted to Center for Veterinary (FDA). March 14, 2000. 94 pp. plus Appendices A and B.

Rach, J.J., M.P. Gaikowski, V.K. Dawson, and R.T. Ramsay. 2000. Pivotal dose titration studies to evaluate the efficacy of hydrogen peroxide to control mortalities associated with external flavobacter infections on cultured fish at selected fish hatcheries. Report submitted to Center for Veterinary (FDA). January 28, 2000. 337 pp.

Rach, J.J., M.P. Gaikowski, V.K. Dawson, and R.T. Ramsay. 2000. Pivotal studies to evaluate the efficacy of hydrogen peroxide to control parasite infestations on cultured fish at selected fish hatcheries. Report submitted to Center for Veterinary (FDA). May 1, 2000. 279 pp.

Lovetro, D. 1999. Product chemistry of hydrogen peroxide. Request submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). July 12, 1999 by Eka Chemicals, Inc.

Lovetro, D. 1999. Request to CVM to write the human food safety section of the FOI summary for hydrogen peroxide. Technical Section submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). November 10, 1999 by Eka Chemicals, Inc.

Hydrogen Peroxide, CVM Response. On January 7, 2000, CVM provided a response to Eka Chemicals, Inc. product chemistry submission indicating that the submission was incomplete and that the sponsor needed to provide more data.

On March 22, 2000, CVM provided a FOI summary for the human food safety of hydrogen peroxide indicating “The human food safety concerns for the use of hydrogen peroxide on all fish and their eggs are satisfied. Neither an acceptable daily intake (ADI), tolerance, withdrawal time, nor regulatory method are assigned”.

On March 17, 2000, CVM accepted a label claim for a previously submitted efficacy technical section that a 15-minute treatment at 500 mg/L with hydrogen peroxide controlled mortalities associated with saprolegniasis on all salmonid eggs.

On March 17, 2000, CVM reviewed a previously submitted target animal safety technical section on fish eggs and did not agree with UMESC’s assessment that hydrogen peroxide administered at 1,000 mg/L for 15 minutes through hatch was safe for all fish species and requested target animal safety studies on one cold water and one warm water fish.

Oxytetracycline, Submissions.

Oeller, M. 1999. Complete package for a supplemental NADA for the marking of all fish by

immersion. Volumes submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA) November 1, 1999 by the CVM/NRSP-7 liaison.

Bernardy, J.A., C. Vue, and M.P. Gaikowski. 2000. Validation of an HPLC method for oxytetracycline in coho salmon and northern pike fillet tissue (Includes data from UMESC study CAP-95-00084-01, Liquid chromatographic determination of oxytetracycline in edible fish fillets from six species of fish). Report submitted to Center for Veterinary Medicine (FDA). July 10, 2000.

Bowker, J.D. 2000. The efficacy of oxytetracycline-medicated feed to control mortality of juvenile steelhead trout *Oncorhynchus mykiss* caused by columnaris, (causative agent *Flavobacterium columnare),* - Coleman Study #01. Report submitted to Center for Veterinary Medicine (FDA). June 12, 2000.

Bowker, J.D. 2000. The efficacy of oxytetracycline-medicated feed to control mortality of juvenile steelhead trout *Oncorhynchus mykiss* caused by columnaris (causative agent *Flavobacterium columnare),* - Coleman Study #02. Report submitted to Center for Veterinary Medicine (FDA). July 14, 2000.

Bowker, J.D. 2000. The efficacy of oxytetracycline-medicated feed to control mortality of fingerling coho salmon *Oncorhynchus kitusch* caused by coldwater disease (causative agent *Flavobacterium psychrophilum*). Report submitted to Center for Veterinary Medicine (FDA). August 14, 2000.

Oxytetracycline, CVM Response. In reviewing an efficacy technical section based on a data call-in submitted in January 1999, CVM indicated on February 1, 2000 that a lack of adequate controls limits the conclusions that can be drawn on the efficacy against *A. hydrophila* in northern pike.

Current Status: See Job No. 1 in Study Plan No. 10 for details on the status of each Technical Section Submission for each IAFWA Project drug.

APPENDIX I: PRODUCTS OF THE PROJECT "APPROVAL OF DRUGS FOR PUBLIC FISH PRODUCTION" IN THE SIXTH ANNUAL REPORTING PERIOD

NADA SUBMISSIONS

Bell, D. 1999. Residue depletion data on AQUI-S™. Report submitted to Center for Veterinary (FDA) by AQUI-S New Zealand LTD. December 1999.

Bernardy, J.A., C. Vue, and M.P. Gaikowski. 2000. Validation of an HPLC method for oxytetracycline in coho salmon and northern pike fillet tissue (includes data from UMESC study CAP-95-00084-01, Liquid chromatographic determination of oxytetracycline in edible fish fillets from six species of fish). Report submitted to Center for Veterinary Medicine (FDA). July 10, 2000.

Bova, A. 1999. Control of furunculosis in salmonids. Five technical sections submitted to CVM. Volumes submitted by Schering-Plough Animal Health to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). September 30, 1999.

Bowker, J. D. 1997. Amended Report: Efficacy of chloramine-T to control to control mortality in apache trout caused by bacterial gill disease associated with flavobacters. Report submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). January 10, 2000.

Bowker, J. D. 1997. Amended Report: Efficacy of chloramine-T to control mortality caused by bacterial gill disease associated with flavobacters in fall chum salmon fingerling. Report submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). January 10, 2000.

Bowker, J. D. 1997. Amended Report: Efficacy of chloramine-T to control mortality caused by bacterial gill disease associated with flavobacters in rainbow trout fingerling. Report submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). January 10, 2000.

Bowker, J. D. 2000. The efficacy of oxytetracycline-medicated feed to control mortality of juvenile steelhead trout *Oncorhynchus mykiss* caused by columnaris, (causative agent *Flavobacterium columnare),* - Coleman Study #01. Report submitted to Center for Veterinary (FDA). June 12, 2000.

Bowker, J. D. 2000. The efficacy of oxytetracycline-medicated feed to control mortality of juvenile steelhead trout *Oncorhynchus mykiss* caused by columnaris (causative agent *Flavobacterium columnare),* - Coleman Study #02. Report submitted to Center for Veterinary (FDA). July 14, 2000.

Bowker, J. D. 2000. The efficacy of oxytetracycline-medicated feed to control mortality of fingerling coho salmon *Oncorhynchus kitusch* caused by coldwater disease (causative agent

Flavobacterium psychrophilum). Report submitted to Center for Veterinary (FDA). August 14, 2000.

Griffin, B.R. and D.L. Strauss. 2000. Supplement to animal safety data for therapeutic use of copper sulfate on food fish. 13 pp. and appendices A-K. Supplement submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). January 10, 2000.

Howe, G.E., M.P. Gaikowski, L.J. Schmidt, and J.J. Rach. 2000. Environmental assessment for the proposed use of hydrogen peroxide in aquaculture for treating external fungal, bacterial, and parasitic diseases of cultured fish. Report and appendices submitted to the Environmental Assessment Team, Center for Veterinary Medicine (FDA). March 14, 2000.

Lovetro, D. 1999. Product chemistry of hydrogen peroxide. Technical Section submitted by Eka Chemicals Inc. to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). July 12, 1999.

Lovetro, D. 1999. Request to CVM to write the human food safety section of the FOI summary for hydrogen peroxide. Technical Section submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). November 10, 1999 by Eka Chemicals, Inc.

Oeller, M. 1999. Complete package for a supplemental NADA for the marking of all fish by immersion. Volumes submitted by NRSP-7 FDA Liaison to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). November 1, 1999.

Rach, J.J., M. P. Gaikowski, V. K. Dawson, and R.T. Ramsay. 2000. Pivotal dose titration studies to evaluate the efficacy of hydrogen peroxide to control mortalities associated with external flavobacter infections on cultured fish at selected fish hatcheries. Report submitted to Center for Veterinary (FDA). January 28, 2000. 337 pp.

Rach, J.J., M. P. Gaikowski, V. K. Dawson, and R.T. Ramsay. 2000. Pivotal studies to evaluate the efficacy of hydrogen peroxide to control parasite infestations on cultured fish at selected fish hatcheries. Report submitted to Center for Veterinary (FDA). May 1, 2000. 279 pp.

Schreier, T. M., V. K. Dawson, and W. H. Gingerich. 1998. Amended summary report: Efficacy of chloramine-T treatments to control mortalities associated with bacterial gill disease on fish. Amended summary report submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). January 10, 2000. 416 pp.

Weddle, M. 1999. Request to CVM to write the human food safety section of the FOI summary for copper sulfate. Request submitted to Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine (FDA). July 15, 1999 by Phelps Dodge Refining Corporation.

STUDY PROTOCOLS

Bernardy, J.A. 1999. Oxytetracycline residue depletion from walleye fillet tissue. UMESC

study protocol CAP-99-00084-07. Approved by the UMESC Center Director. November 8, 1999. 74 pp.

Gaikowski, M.P. 1999. Toxicity assessment of chloramine-T to cool- and warm water fish. UMESC study protocol CAP-99-CLT-01. Approved by the UMESC Center Director. June 1, 1999. 36 pp.

Gaikowski, M.P. and J.J. Rach. 2000. Study protocol for a compassionate aquaculture investigational new animal drug exemption (INAD #10-023). "Clinical field trials to determine the efficacy of Perox-Aid ™ to control or prevent mortalities associated with saprolegniasis in a variety of cultured fish and their eggs." Submitted to CVM. March 3, 2000. 34 pp.

Gaikowski, M.P. and J.J. Rach. 2000. Study protocol for a compassionate aquaculture investigational new animal drug exemption (INAD #10-023). "Clinical field trials to determine the efficacy of Perox-Aid™ to control or prevent mortalities associated with bacterial gill disease and external flavobacterial infections in a variety of cultured fish species." Submitted to CVM. April 18, 2000. 34 pp.

Gaikowski, M.P. and J.J. Rach. 2000. Study protocol for a compassionate aquaculture investigational new animal drug exemption (INAD #10-023). "Clinical field trials to determine the efficacy of Perox-Aid™ to control or prevent external parasite infections in a variety of cultured fish species." Submitted to CVM. April 18, 2000. 30 pp.

Meinertz, J.R. 1999. Development of a regulatory method for p-TSA in the edible fillet tissue of rainbow trout: evaluation of readiness to perform and bridging with a previously reported method to determine chloramine-T and p-TSA concentrations in rainbow trout tissue. UMESC study protocol CAP-99-PTSA-01. Approved by the UMESC Center Director. October 27, 1999. 19 pp.

Meinertz, J.R. 2000. Development of a regulatory method for p-TSA in the edible fillet tissue of channel catfish and walleye; evaluation of readiness to perform. UMESC study protocol CAP-00-PTSA-02. Approved by the UMESC Center Director. May 19, 2000. 16 pp.

Rach, J. J. 1999. Evaluation of the efficacy of the fish anesthetic AQUI-S^{TM}. UMESC Study Protocol Number CAP-99-00100-03. Approved by the UMESC Director. July 1999. 26 pp.

Rach, J. J. 1999. Pivotal dose-titration studies to evaluate the efficacy of chloramine-T to control mortalities associated with external flavobacter infections on cultured fish at selected fish hatcheries. UMESC Study Protocol Number CAP-99-00057-03. Approved by the UMESC Director. July 1999. 25 pp.

PUBLICATIONS

Gaikowski, M.P., J.J. Rach, and R.T. Ramsay. 1999. Acute toxicity of hydrogen peroxide treatments to selected life stages of cold-, cool-, and warm water fishes. Aquaculture

178:191-207

Griffin, B.R., R.A. Schnick, and W.H. Gingerich. 2000. Update on the Federal-State Aquaculture Drug Approval Project. Aquaculture Magazine 26(3):56-58.

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

Rach J. J., M. P. Gaikowski, and R. T. Ramsay. In Press. Efficacy of hydrogen peroxide to control parasitic infestations on hatchery reared fish. Journal of Aquatic Animal Health.

Rach, J.J. and R.T. Ramsay. 2000. Analytical verification of waterborne chemical treatment regimens in hatchery raceways. North American Journal of Aquaculture 62:60-66.

Schnick, R.A., and P. Smith. 1999. International harmonisation of antibacterial agent approvals and susceptibility testing. EAFP Bulletin 19(6):293-294.

Schnick, R.A. 2000. Efficacy data needed for high priority aquaculture drugs. American Fisheries Society Fish Health Newsletter 28(2):3.

Schnick, R.A. In press. International harmonization of antibacterial sensitivity determination for aquaculture drugs. Aquaculture.

Stehly, G.R., J.R. Meinertz, and W.H. Gingerich. 2000. Effects of temperature on the elimination of benzocaine and acetylated benzocaine residues from the edible fillet of rainbow trout (Oncorhynchus mykiss). Food Additives and Contaminants 17:387-392.

SPECIAL REPORTS

Gingerich, W.H., G.R. Stehly, V.K. Dawson, M.P. Gaikowski, G.E. Howe, J.R. Meinertz, J.J. Rach, R.A. Schnick, and B.R. Griffin. 1999. Approval of Drugs for Public Fish Production: Fifth annual report of progress [performance period: July 1, 1998 to June 30, 1999]. Biological Resources Division, USGS, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin. August 29, 1999. 45 pp.

Gingerich, W.H., G.R. Stehly, V.K. Dawson, M.P. Gaikowski, G.E. Howe, JR. Meinertz, J.J. Rach, R.A. Schnick, and B.R. Griffin. 2000. Approval of Drugs for Public Fish Production: Sixth midyear report of progress [performance period: July 1, 1999 to December 31, 1999]. Biological Resources Division, USGS, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin. January 28, 2000. 39 pp.

Gingerich,, W.H., R.A. Schnick, and B.R. Griffin. 2000. Proposed Work Plan for Project Year 7. Submitted to Federal Aid Coordinators of states participating in the Approval of Drugs for Public Fish Production partnership project. May 25, 2000. 10 pp.

Gingerich,, W.H., R.A. Schnick, and B.R. Griffin. 2000. Amended grant proposal for Project Years 7 and 8. Submitted to Federal Aid Coordinators of states participating in the Approval of Drugs for Public Fish Production partnership project. May 25, 2000 14 pp.

Schnick, R.A. 1999. Status of IAFWA Project drugs in August 1999. Submitted to Mike Gibson, Chair, IAFWA Drug Approval Oversight Subcommittee, Hot Springs, Arkansas. August 10, 1999. 2 pp.

Schnick, R.A. 1999. Status of the aquaculture drug approval process in August 1999. Submitted to Website. August 19, 1999. 5 pp.

Schnick, R.A. 1999. List of drugs, sponsors, INAD/NADA numbers, and dates (August 24, 1999). Submitted to Website, Washington, DC. August 24, 1999. 3 pp.

Schnick, R.A. 1999. Minutes to the MUMS Meeting with CVM, September 8, 1999. Submitted to Randy MacMillan, Chairman of the MUMS Coalition, Buhl, Idaho, for transmission to the attendees and CVM for comment. September 13, 1999. 6 pp.

Schnick, R.A. 1999. MUMS provisions summary as of September 9, 1999. Submitted to Mike Gibson, Chair, IAFWA Drug Approval Oversight Subcommittee, Hot Springs, Arkansas. September 15, 1999. 2 pp.

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CONTRACT PROPOSALS

None

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