

## **First Annual Report of Progress**

Performance Period: July 1, 1994 to June 30, 1995

**Approval of Drugs for Public Fish Production**

A project of the

**International Association of Fish and Wildlife Agencies (IAFWA)**

by

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## **INTRODUCTION**

A partnership was developed between the National Biological Service (NBS), the U.S. Fish and Wildlife Service (FWS), and the International Association of Fish and Wildlife Agencies (IAFWA) on behalf of state fish and wildlife agencies to conduct research for the approval of aquaculture drugs by the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM).

Research to gain new animal drug application (NADA) approvals or extensions of approvals for eight drugs identified as high priority by the states and to establish crop grouping was initiated July 1, 1994 and will extend until June 30, 1999. During that 5-year period, at least 39 states will contribute a total of \$3.9 million, NBS's Upper Mississippi Science Center (UMSC, formerly National Fisheries Research Center, La Crosse, Wisconsin) will provide \$4.3 million, and the Fish Farming Experimental Laboratory, Stuttgart, Arkansas (FFEL) will provide some base funding toward: a) development of data under GLP provisions to gain CVM approval of NADAs for use in aquaculture for the following essential drugs; 1) extension of formalin to other species, 2) expansion to other diseases and extension to other species for oxytetracycline, 3) copper sulfate, 4) chloramine-T, 5) sarafloxacin hydrochloride (sarafloxacin), 6) potassium permanganate, and 7) benzocaine; b) development of safety and efficacy data to delineate concentrations of hydrogen peroxide for control of fungi on various fish species and eggs and efficacy data to expand the low regulatory priority status of hydrogen peroxide for potential control of external parasitic infestations and external bacterial infections on freshwater fishes; and c) development of research information to allow acceptance of a crop grouping concept by CVM. Data will be generated to allow CVM to assess whether a few selected fish species can be used as surrogates for all or most of the cultured fishes in the United States.

The development of aquaculture NADAs through the IAFWA project is contingent upon continued base funding at UMSC of \$867,000 per year designated for chemical and drug registration activities, contribution of some base funding from FFEL, and state funding of at least \$780,000 per year under a five-year agreement through the IAFWA. A portion of the state contribution (\$50,000 to \$160,000 per year) and FFEL base funding will be used by FFEL for research on copper sulfate and potassium permanganate. The remainder of the state funding (\$620,000 to \$655,000 per year) and UMSC base funding will be used directly by UMSC to conduct internal studies or to contract for external studies to achieve original or extended NADAs. The proposed studies to be conducted by UMSC and FFEL are estimated to cost \$8.9 million and only \$8.2 million is available, resulting in a need for an additional \$700,000.

**HIGHLIGHTS OF PROGRESS  
(JULY 1, 1994 TO JUNE 30, 1995)**

- **Formalin**: A compassionate Investigational New Animal Drug (INAD) exemption to allow extended use and efficacy evaluation of formalin as a fungicide at public aquaculture facilities was developed, approved, implemented, and revised. Efficacy data generated will be used to support extension of the current formalin label to include fish and the eggs of warmwater and other coolwater species.
- **Formalin**: A Target Animal Safety Study on the toxicity of formalin to warm- and coolwater fish eggs has been completed and the final report will soon be submitted to CVM to support extension of the current formalin label as a fungicide to include the eggs of warmwater and other coolwater species.
- **Copper Sulfate**: A study was completed to determine the accumulation of copper in channel catfish exposed to copper sulfate. This data indicates that copper does not accumulate in fish tissue and will be used to fulfill the residue chemistry portion of the human food safety data requirements for a New Animal Drug Application (NADA) for copper sulfate.
- **Chloramine-T**: A compassionate INAD agreement to allow use and efficacy evaluation of chloramine-T to control bacterial gill disease and flexibacteriosis at public aquaculture facilities was developed, approved, and implemented.
- **Chloramine-T**: A second study report on metabolism and elimination of chloramine-T residues by rainbow trout was submitted to FDA to address questions raised by the Center for Veterinary Medicine (CVM) from a previous study and to supplement a previous investigation of total residue depletion.
- **Sarafloxacin**: Research for the approval of sarafloxacin was postponed pending resolution of questions on bacterial resistance with fluoroquinolones. Research dollars and effort from this study were transferred to expand and extend the label for oxytetracycline.

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- **Benzocaine:** A study to evaluate the effects of temperature on the accumulation and loss of benzocaine and acetylated benzocaine, a major metabolite of benzocaine, in fillet tissue taken from channel catfish exposed to anesthetizing concentrations of benzocaine was completed as part of an interagency agreement with CVM's Office of Science. The study will be submitted to CVM in September and will supplement residue chemistry data to support the NADA application for benzocaine as an anesthetic/sedative in cultured freshwater fish.
- **Hydrogen Peroxide:** A Target Animal Safety Study on the toxicity of hydrogen peroxide to representative warm- and coolwater fish eggs has been completed and will be used to support an amendment to the current low regulatory priority ruling on hydrogen peroxide to allow its use at concentrations up to 1,000 L/L (ppm) on fish eggs.
- **Crop Grouping:** A research plan to allow validation of the Crop Grouping concept was developed, presented at a national forum in September 1994, and the plan was evaluated for approach and depth by a review team of independent peer research scientists in May 1995.
- **Crop Grouping:** Two research work orders were developed with Dr. William Hayton, College of Pharmacy and Pharmaceutical Sciences, The Ohio State University, to undertake the benzocaine portion of the Crop Grouping study plan and two doctoral candidate students were hired to conduct the research.
- **Negotiations and Contract Coordination:** The human food safety data requirements for benzocaine were determined and an Interagency Agreement was drafted to have CVM's Office of Science administer two 90-day mammalian feeding studies for benzocaine.
- **Negotiations and Contract Coordination:** Two meetings were held with INAD coordinators to discuss collaborative efforts for INAD studies, implement the format and parameters needed for NADAs, and troubleshoot and streamline the INAD process.

## **SUMMARY OF PROGRESS BY RESEARCH STUDY PLAN**

A summary follows on the progress made during the period from July 1, 1994 to June 30, 1995 for each of the ten Research Study Plans in the IAFWA Project.

### **STUDY NO. 1: EXTENSION 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 fungal infections on eggs and adults of publicly cultured freshwater fish.

**Job No. 1:** Supervision of the formalin Compassionate Investigational New Animal Drug (INAD) permit.

**Progress:** A protocol for the FWS's INAD for formalin to support the extended use of formalin as a fungicide on fish eggs cultured on public hatcheries was written by staff at UMSC and submitted to CVM for review. The protocol was approved October 14, 1994 and the INAD study began November 1, 1994. The sponsorship of the FWS INAD is now under administration of the FWS. Several workshops and coordination meetings have been conducted involving FWS's INAD coordinator and study monitors and UMSC NADA coordinators. Formalin treatments have been administered under the INAD and data from the first six months have been gathered and discussed. Necessary revisions to the study protocol have been identified and will be implemented before the second year of treatments. Efforts are underway to identify hatcheries to provide high quality pivotal data for the new NADA. Representatives of UMSC met on October 28, 1994 with the coordinator for the Western Regional INAD Project to coordinate data transfer to UMSC for the formalin NADA. The UMSC has reviewed and commented on the study protocols for the western project. The UMSC has contacted all formalin (fungicide) INAD holders and discussed the eventual collection, pooling, and analysis of all data to support the new NADA. Sample data spreadsheets have been designed and distributed to INAD holders.

**Current Status:** The formalin (fungicide) INAD will be revised and implemented for a second year. CVM is currently reviewing a data package on formalin that could result in a label extension to include fungicide use on fish and eggs of warmwater and other coolwater fish in 1996.

**Job No. 2:** Conduct controlled laboratory studies on a variety of fish species to evaluate the efficacy of formalin as a fungicide on cultured freshwater fish and their eggs.

**Progress:** Formalin is currently approved as a fungicide for use on salmonid and esocid eggs. Research underway at UMSC is necessary to support the extension of the formalin NADA to include fungicidal treatment of eggs of additional species of cultured freshwater fish. Formalin treatments on a variety of fish eggs effectively controlled fungus (*Saprolegnia parasitica*) when 10% of the eggs were infected.

The UMSC has drafted a protocol and initiated a study to induce fungal infections on adult fish. If the UMSC is successful in inducing fungal infections on adult fish, the efficacy of formalin as a fungicide will be evaluated. Preliminary studies to induce fungal infections on channel catfish and administer formalin treatments have been conducted.

**Current Status:** Efficacy research is on-going and will continue for the remainder of this year. A protocol will be developed for treating infected fish with formalin.

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

**Progress:** Scientists at the UMSC have completed formalin target animal safety studies on the eggs of representative warm- and coolwater fish species. Eggs were treated with multiples (1X, 3X, 5X) of the standard label-recommended formalin treatment. The margin of safety was considerable for walleye, carp, and white sucker as was evident by the relatively strong hatches observed even for eggs treated with three times the standard treatment concentration and duration. The margin of safety was slightly less for channel catfish with all eggs hatching even after standard treatments three times the normal duration. Results did not define a clear margin of safety for lake sturgeon. For all species tested, the hatching success after standard formalin treatments for three times the normal duration was greater than that observed for control groups. Most control eggs became severely infected with fungus while nearly all eggs receiving formalin treatments were uninfected.

**Current Status:** The final report on the completed Target Animal Safety Study is being prepared and will soon be submitted to CVM. This data will support extension of the current formalin label to include fungicide treatment of eggs of warmwater and other coolwater species.

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

**Objectives:** To extend the feed additive label for treatment of certain bacterial diseases on cool- and warmwater fish species of importance to public fish production and to cover marking of fish species not covered by the current label. To expand the feed additive label for control of flexibacteriosis on cold-, cool-, and warmwater fishes.

**Job No. 1:** Develop efficacy data or determine if current data is adequate on oxytetracycline to expand the label.

**Progress:** Personnel of the UMSC discussed coordination of data acquisition from INADs for oxytetracycline with representatives of the FWS and the Western Regional INAD project. Included were two meetings with the FWS group to discuss problems associated with the INADs and possible solutions. Some of the early data from these studies have been reviewed. Quality of data obtained in the first year will be critical in identifying sites for the collection of pivotal data.

**Current status:** Data collection from INADs will continue and will be used to determine hatcheries capable of providing pivotal data for the efficacy of oxytetracycline.

**Job No. 2:** Develop residue chemistry data on oxytetracycline in cool- and warmwater fish.

**Progress:** A protocol "Analytical Method Development Studies to Expand and Extend the Use of Oxytetracycline in Public Fish Production" was written and approved. Preliminary work on this protocol to adapt an analytical method for oxytetracycline in salmon has been completed. Characteristics of the accuracy and precision of the method in edible tissue of several species of fish (cold-, cool-, and warmwater) will be tested when the method is finalized. Before the analytical method can be used, bridging data to show the similarity of the analytical method to the microbial inhibition assay on which the current drug approval is based, must be demonstrated. Future work will address development of information to amend the label to include treatment of coldwater species below 9 C.

**Current Status:** The method is under refinement for ease of use and increased sensitivity. The method will be used for determination of residue depletion which is expected for expansion and extension of the current label.

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**Job No. 3:** Develop target animal safety data on oxytetracycline in cool- and warmwater species.

**Progress:** No activity during July 1, 1994 to June 30, 1995.

**Current Status:** Testing will begin with the development of efficacy data and when other jobs are completed.

**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.

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

**Progress:** A research protocol entitled "Accumulation of Copper in Edible Muscle of Channel Catfish (*Ictalurus punctatus*) Following Exposure to Water Borne Copper Sulfate", was prepared and submitted to the FDA National Center for Toxicological Research (NCTR) and to CVM for review. The protocol was approved by both NCTR and CVM (dated 9/30/94).

**Current Status:** The Study Protocol that was approved September 9, 1994 was used to conduct research under Job No. 2.

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

**Progress:** The study was initiated November 9, 1994. The conditions of the study in the approved protocol kept three researchers actively attending to the study until April 11, 1995. Exposures as well as water and tissue residue analyses are complete and compilation, calculations, and evaluation needed to prepare the report for NCTR and CVM are underway. Preliminary conclusions are that exposures of channel catfish to high concentrations of water borne copper sulfate does not elevate residual copper in edible flesh.

**Current Status:** The final report of the study is expected to be completed and submitted to NCTR and CVM by November 1, 1995.

**Job No. 3:** Prepare an environmental assessment of the fate and effects of release of copper sulfate treated water.

**Progress:** An assessment of the fate and effects of release of copper sulfate treated water has been completed and is now undergoing revision to meet CVM organizational guidelines.

**Current Status:** Environmental assessment is being revised to meet CVM requirements.

**Job No. 4:** 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.

**Progress and Current Status:** No activity during July 1, 1994 to June 30, 1995.

**STUDY NUMBER 4: APPROVAL OF CHLORAMINE-T TO CONTROL BACTERIAL GILL DISEASE ON SALMONIDS AND FLEXIBACTERIOSIS ON COLD-, COOL-, AND WARMWATER FISH SPECIES.**

**Objectives:** To 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 flexibacteriosis on cultured freshwater fish.

**Job No. 1:** Conduct a mutagenicity study in support of the approval of chloramine-T as a drug.

**Progress:** No activity during July 1, 1994 to June 30, 1995.

**Current Status:** CVM must review mammalian toxicology data for chloramine-T before a decision is made whether a mutagenicity study is required for the drug approval submission.

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

**Progress:** No activity during July 1, 1994 to June 30, 1995.

**Current Status:** CVM must review existing environmental fate and effects data for chloramine-T before a decision is made which environmental studies are required for the drug approval submission.

**Job No. 3:** Supervision, data collection and analysis of efficacy data collected from the FWS compassionate INAD for use of chloramine-T on FWS hatcheries.

**Progress:** A protocol for the FWS's INAD for chloramine-T to support its use for treatment of BGD and flexibacteriosis on fish raised on public aquaculture facilities was written by UMSC personnel and submitted to CVM for review. The protocol has been approved and the INAD study has been initiated at 52 hatcheries. The protocol has been used as a template for other INAD applications. Several workshops and coordination meetings have been conducted involving FWS's study director, study monitors, INAD coordinators and UMSC NADA coordinators. Evaluations of preliminary data that are being submitted to the INAD coordinators suggests there may be some problems obtaining pivotal data that can be used to support the eventual NADA submission. Some of the problems include the lack of controls, replicate treatments, long term monitoring of fish lots following treatments, and confirmation of disease both before and after treatments. Some of these problems are being addressed by closer coordination by study monitors and by revisions of the protocol.

**Current Status:** The protocol has been approved and the INAD study has been initiated at 52 hatcheries. Some of the preliminary data from these INAD's are beginning to be submitted to the INAD coordinators. The protocol for the chloramine-T INAD to be used during the second year of the INAD program has been revised based on the recent review of INAD data submissions.

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

**Progress:** A report on total residue depletion and metabolism using radiolabelled chloramine-T previously had been submitted by UMSC scientists to CVM, FDA for review. Based on review comments from CVM for the initial study, a second study was undertaken to address unresolved issues. The results of the second study were submitted to CVM in January 1995.

**Current Status:** To date, CVM has not responded to information contained in the second study. Once CVM responds to the data contained in the second study, it will be clear whether additional information will be required or whether sufficient information exists to assign a marker residue for withdrawal studies. If a marker residue is assigned, a method for it will be developed and validation studies will be conducted in a series of laboratories. After successful completion of a validation of the method, a marker residue depletion study will be conducted using the method. The results will be submitted to support the time of withdrawal of treatment from chloramine-T.

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

**Progress:** No activity during July 1, 1994 to June 30, 1995.

**Current Status:** Target animal safety studies are planned for rainbow trout and other salmonids during the second year of this project. No target animal safety protocols currently exist for chloramine-T exposure to juvenile and/or adult fish. Methods are currently being developed to assess how best to resolve questions related to the safety of chloramine-T in sensitive fish species. The work is being complicated by changes in chloramine-T toxicity with changes in water pH, hardness and alkalinity. A battery of physiological, anatomical, and biochemical responses are being considered as potential discriminators of toxicity to target animals.

#### **STUDY NUMBER 5: APPROVAL OF SARAFLOXACIN HYDROCHLORIDE AS A DRUG TO CONTROL FLEXIBACTERIOSIS AND FURUNCULOSIS IN FRESHWATER FISH.**

**Objectives:** To develop efficacy, target animal safety, and total residue and metabolism data required for the use of sarafloxacin in the control of furunculosis and flexibacteriosis in freshwater cold-, cool-, and warmwater fish. Expansion of the label to include other diseases is beyond the scope of the current protocol.

**Progress:** In November 1994, CVM decided that the use of all fluoroquinolone drugs would be restricted. The decision was made because of the increasing incidence of bacterial resistance developing among common human bacterial pathogens to the variety of medicinal drugs currently approved. The decision has forced UMSC to revise its plan to attempt to gain approval for the use of sarafloxacin in aquaculture. In response to this decision, UMSC has shifted resources and dollars formerly in the sarafloxacin study into attempts to extend and expand the applications of oxytetracycline in freshwater aquaculture. See Study No. 2 for details on the current efforts.

**Current Status:** While UMSC has temporarily discontinued attempts to aggressively gain drug approvals for sarafloxacin in public aquaculture, it is possible that CVM may remove the restrictive moratorium on the use of fluoroquinolones for aquaculture uses at a later date. For this reason, UMSC will continue to use sarafloxacin as a model chemical to define comparative pharmacokinetics and metabolism of an oral drug as part of the research initiative for the crop grouping portion of the project.

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

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

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

**Progress:** A research protocol entitled "Accumulation of Manganese in Edible Muscle of Channel Catfish (*Ictalurus punctatus*) Following Exposure to Water Borne Potassium Permanganate", was prepared and submitted to NCTR and CVM for review. The protocol has been reviewed and approved pending revision. Revisions were completed and the revised protocol was returned to NCTR and CVM on August 16, 1995.

Preliminary range finding studies were completed to determine more accurately the tolerance of channel catfish to potassium permanganate before submitting exposure levels in a protocol for CVM approval. These are pilot exposure/tolerance tests to provide guidelines for the study and are not intended to gather data on tissue residues of manganese.

Contacts have been made with Clear Springs Foods, Inc. (Buhl, Idaho) to expose rainbow trout to potassium permanganate and have the samples analyzed through FFEL.

**Current Status:** The Study Protocol has been approved by CVM.

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

**Progress:** The study is scheduled to start on October 1, 1995. Fish of known manganese exposure have been in culture since hatching in May 1994. Fish for the study are now in the 250 to 400 g weight range and need to be in the 400 to 600 g range for the study.

**Current Status:** The residue study on manganese will start October 1, 1995 according to the approved study protocol.

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

**Progress and Current Status:** A preliminary search for literature from five data bases has been completed and a reference file containing 400 entries are being reviewed.

**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 and Current Status:** No activity during July 1, 1994 to June 30, 1995.

### **STUDY NUMBER 7: APPROVAL OF BENZOCAINE AS AN ANESTHETIC AND SEDATIVE FOR FISH.**

**Objectives:** To develop efficacy, target animal safety, and residue depletion data required for the approval of benzocaine as an anesthetic/sedative with a short withdrawal time for several species of freshwater fish.

**Job No. 1:** Development of a compassionate INAD request to evaluate benzocaine as an anesthetic/sedative for fish cultured on public fish hatcheries.

**Progress:** No activity during July 1, 1994 to June 30, 1995.

**Current Status:** Lack of progress in this job is directly related to the lack of a sponsor for benzocaine. A compassionate INAD will not be initiated until a sponsor is identified who can supply the test chemical.

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**Job No. 2:** Residue chemistry studies in freshwater fish to support the use of benzocaine as an anesthetic/sedative.

**Progress:** Methods have been developed, based on previous studies of total residue depletion and metabolism with radiolabelled benzocaine, to extract, separate and quantify benzocaine and its principle metabolite, acetylated benzocaine, from fillet tissue of exposed rainbow trout and channel catfish. Separate studies were conducted at UMSC to define the effects of temperature on the loss of benzocaine and acetylated-benzocaine in channel catfish and rainbow trout. Channel catfish were exposed at the Southeastern Fish Cultural Laboratory, Marion, AL. Center scientists at UMSC worked closely with reviewers at CVM to develop protocols for both studies. The study to address the effects of temperature on the loss of benzocaine in fillet tissues of channel catfish was conducted under an Interagency Agreement with CVM's Office of Science.

**Current Status:** The residue chemistry study for channel catfish acclimated to two temperatures has been completed and will be submitted to the Office of Science in September 1995. The residue chemistry study for rainbow trout continues and should be completed at the end of the calendar year. Methods developed for rainbow trout and channel catfish should be easily transferrable to other species. Additional research requirements for the residue chemistry of benzocaine will be determined by CVM.

**Job No. 3:** Target animal safety studies in rainbow trout and a second species (cool- or warmwater) to support the approval of benzocaine as an anesthetic/sedative.

**Progress:** No activity during July 1, 1994 to June 30, 1995.

**Current Status:** Target animal safety studies will be initiated after similar studies are completed for chloramine-T, formalin, and hydrogen peroxide.

**Job No. 4:** Mutagenicity testing in support of the approval of benzocaine as an anesthetic/sedative for all fish on public production.

**Progress:** Based on discussions with CVM officials at a meeting on November 15, 1994, CVM will not require mutagenicity testing data to support the approval of benzocaine as an anesthetic/sedative in cultured freshwater fish.

**Current Status:** No further activity required because data requirements have been met.

**Job No. 5:** Subacute mammalian toxicity studies in support of the approval of benzocaine as an anesthetic/sedative for all fish in public production.

**Progress:** Based on discussions of November 15, 1994 with CVM officials, only 90- day rodent and 90-day non-rodent feeding studies will be required.

**Current Status:** The UMSC staff drafted an Interagency Agreement with CVM's Office of Science to contract for these studies in FY-1996.

**Job No. 6:** Environmental fate and effects studies in support of the approval of benzocaine as an anesthetic/sedative for all fish in public production.

**Progress:** No activity during July 1, 1994 to June 30, 1995.

**Current Status:** Based on discussions of November 1994 with CVM officials, the UMSC was encouraged to provide environmental information on benzocaine as soon as feasible to the CVM

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Environmental Sciences Staff for review and recommendations for a draft environmental assessment. When a sponsor for benzocaine has been identified, they may be able to provide this information.

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

**Objectives:** To develop efficacy and target animal safety data to provide fish culturists with effective, safe treatment regimes for hydrogen peroxide to control fungal infections on fish and fish eggs and, potentially, for controlling external parasitic infestations and external bacterial infections on freshwater fish.

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

**Progress:** Studies at UMSC indicate that hydrogen peroxide is as effective as formalin for the control of fungus on incubating eggs. The primary decomposition products of hydrogen peroxide (oxygen and water) are considered to be environmentally compatible. The CVM has accepted a petition from UMSC to classify hydrogen peroxide as a low regulatory priority (LRP) drug (for enforcement purposes) when used at concentrations up to 500 L/L to control fungi on all species and life stages of fish. A protocol is being developed to delineate the efficacy of hydrogen peroxide for controlling fungal infections on juvenile and adult fish.

A protocol has been developed and approved for treating salmonid eggs with hydrogen peroxide. Rainbow trout, lake trout, and brown trout eggs have been tested and additional tests with Atlantic salmon are planned. The efficacy of hydrogen peroxide treatments to rainbow trout eggs and fry was evaluated and the data indicated that daily treatments could be safely applied through hatching at concentrations up to 1,000 L/L.

Staff at the UMSC have drafted a protocol and initiated a study to induce fungal infections on fish. If this study is successful, infected fish will be used to evaluate the efficacy of hydrogen peroxide as a fungicide. Preliminary studies to induce fungal infections on channel catfish and administer treatments were successful.

**Current Status:** Efficacy studies will continue. Hydrogen peroxide appears to be effective in controlling fungal infections on fish and their eggs. Data from these studies will be used to support an amendment to the current LRP on hydrogen peroxide to allow use of concentrations up to 1,000 L/L on fish eggs.

**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:** Researchers at UMSC have drafted a protocol entitled "Evaluation of the Efficacy and Safety of Hydrogen Peroxide Exposures to Fish at Fish Hatcheries." This protocol outlines a two-year plan to conduct on-site hydrogen peroxide treatments on diseased fish at federal or state hatcheries. Studies will also be conducted on diseased fish transported to the UMSC.

Hydrogen peroxide treatments have been tested on largemouth bass diagnosed with bacterial gill disease and a protozoan infestation. Fish were treated on four occasions, every-other-day, for one hour with hydrogen peroxide concentrations ranging from 50 to 200 L/L. Treatments of 100 L/L and greater were successful in controlling the bacterial gill disease; however, protozoans were still present on the gills.

**Current Status:** Efficacy studies will continue throughout this coming year. Preliminary results indicate that hydrogen peroxide may be effective in controlling superficial bacterial infections and parasitic infestations in freshwater fish.

**Job No. 3:** Conduct target animal safety studies on adult fish and fish eggs with hydrogen peroxide in support of its intended use as an antifungal agent and therapeutic to control external parasitic infestations and external bacterial infections on cultured freshwater fish.

**Progress:** Target Animal Safety Studies on the eggs of walleye, white sucker, northern pike, channel catfish, common carp, lake sturgeon, and paddlefish were conducted. Treatments of hydrogen peroxide of 1,000 L/L for 15 min were safe for most species of fish eggs.

Toxicity tests were also conducted with fish to determine: 1) the sensitivity of six fish species to hydrogen peroxide treatments, 2) the sensitivity of various life stages of rainbow trout to hydrogen peroxide treatments and 3) the effect of temperature on the sensitivity of fish to hydrogen peroxide. The data indicated that toxicity varied between species and was dependent on the health of test fish. Hydrogen peroxide was more toxic to larger fish and this factor must be considered when treatments are administered. The toxicity of hydrogen peroxide increases significantly with temperature.

**Current Status:** Report preparation on completed safety studies will continue this coming year. Hydrogen peroxide appears to be safe for use on a wide variety of fish species and life stages. Data from these studies will be used to support amending the current LRP status of hydrogen peroxide to allow use of concentrations up to 1,000 L/L on fish eggs.

#### **STUDY NUMBER 9: DEVELOPMENT AND EXECUTION OF STUDIES TO ADDRESS THE CONCEPT OF CROP GROUPING.**

**Objectives:** 1) To 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 and 2) to 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.

**General Progress:** Several accomplishments unrelated to the identified jobs were achieved in this study during the initiation period of the project. Accomplishments include: 1) Development of an experimental approach to the issue of crop grouping in comparative metabolism and pharmacokinetics to address the issues of residue chemistry; 2) Presentation of the approach at a national forum entitled "Drugs in Aquaculture: Current Status-Future Goals" sponsored by US Department of Agriculture, Food and Drug Administration, and National Research Support Project No. 7 at Bethesda, MD, September, 1994; 3) a peer review of the research study plan was conducted in May 1995 by Dr. J. Lech (comparative metabolism), Medical College of Wisconsin; Dr. S. Sower (comparative physiology), University of New Hampshire; Mr. Harvey Clewell (pharmacokinetics), ICS Kaiser International; and Dr. L. Ventura (oversight), CVM.

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

**Progress:** The crop grouping concept was presented to a panel of peer experts. Work to be completed and methods of data analysis for this study were also presented. The panel delivered a report of recommendations for this project that are being incorporated into research study plans. No laboratory activity during July 1, 1994 to June 30, 1995.

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**Current Status:** A protocol for initial studies is nearing completion and will be initiated when personnel currently working on benzocaine residue studies in rainbow trout are available.

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

**Progress:** No activity during July 1, 1994 to June 30, 1995.

**Current Status:** Work on this job will be initiated after more information on the pharmacokinetics and metabolism of sarafloxacin in rainbow trout and channel catfish are developed by UMSC.

**Job 3:** Develop comparative pharmacokinetics and metabolism data for benzocaine in rainbow trout and channel catfish.

**Progress:** The first of two research work orders was initiated with Dr. William Hayton, College of Pharmacy and Pharmaceutical Sciences, The Ohio State University, to undertake research related to pharmacokinetics and metabolism of benzocaine in cultured freshwater fish. Ms. Kelli Clark, a doctoral candidate with Dr. Hayton was hired to act as a principle researcher on the project. A high performance liquid chromatograph was purchased, set up, and is operational. Work has been initiated to develop methods for isolation, separation and quantification of major metabolites of benzocaine in trout and catfish. Pilot studies to evaluate the pharmacokinetics of benzocaine in rainbow trout after intravascular administration were initiated. Physiologically-based pharmacokinetic models for rainbow trout and channel catfish are being developed.

**Current Status:** Data from this job will continue to be collected and developed into a database that can be accessed for use in development of pharmacokinetic models and for the establishment of metabolite residues in trout and catfish.

**Job 4:** Develop comparative pharmacokinetics and metabolism data for benzocaine in phylogenetically diverse aquaculture species to support or refute a crop grouping concept for fish.

**Progress:** A second research work order was initiated with Dr. William Hayton, College of Pharmacy and Pharmaceutical Sciences, The Ohio State University, to undertake research under this job function.

**Current Status:** Ms. Kirsten Engelfried, a doctoral candidate with Dr. Hayton, has been hired for the project and will begin work in September 1995.

## **STUDY NO. 10: NEGOTIATIONS AND CONTRACT COORDINATION**

**Objectives:** To ensure that all data required by CVM for approval through NADAs are developed for the seven priority drugs in a timely, logical, and efficient manner. To coordinate the administration of all contracts by CVM's Office of Science to ensure efficiency, timelessness, and acceptability of data to CVM.

To track and report the progress of all of studies and ensure that they are proceeding for approval in a timely, logical, and efficient manner. To assemble and submit NADAs for approval to CVM.

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

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**Progress:** Personnel of the UMSC met with CVM personnel on November 15, 1994 to determine the data requirements and status for benzocaine, MS-222, sarafloxacin, and oxytetracycline.

**Current Status:** Based on the discussions mentioned above, UMSC personnel began working with CVM's Office of Science to develop an outside contract to generate data on two 90-day feeding studies using benzocaine, dropped MS-222 from further efforts to reduce the 21-day withdrawal time, dropped sarafloxacin temporarily until its status is clarified with CVM, and accelerated research activities on oxytetracycline.

**Job No. 2:** Coordinate the administration of contracts by CVM's Office of Science.

**Progress:** Personnel of the UMSC developed a draft Interagency Agreement (IAG) with CVM's Office of Science that is in review. The Office of Science requested cost and project proposals from NCTR for mammalian studies on benzocaine that were identified as needed for approval.

**Current Status:** The UMSC plans to finalize the IAG with the Office of Science and initiate studies on benzocaine.

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

**Progress:** Staff from the UMSC have established a system to track the use of funds and progress on each Study Plan for the IAFWA project. A mid-year report of progress was written and submitted in January 1995 to Doug Hanson of the South Dakota Department of Natural Resources and Chairman of the Aquaculture Committee, IAFWA. Jane Gofus was hired as an assistant to help the Quality Assurance Officer and the Section of Chemistry and Physiology with their roles and responsibilities with the IAFWA Project. Presentations have been made to various groups on IAFWA Project activities.

**Current Status:** Appropriate progress reports have been and will continue to be presented to the IAFWA Project participants and stake holders.

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

**Progress:** Personnel of the UMSC met with coordinators for the Western Regional INAD Project and FWS INAD Project along with the FWS Monitors. The purpose of the two meetings was to standardize the reporting format for data and ensure a timely and organized collection of all efficacy data to support a NADA.

A request was submitted to CVM General Counsel to determine whether exclusivity could be extended for minor use drugs to give companies more time to recoup their investment on aquaculture drugs.

**Current Status:** Personnel of the UMSC plan to initiate a compassionate INAD on benzocaine. A meeting for INAD Coordinators will be held November 1-2, 1995 at CVM to coordinate data generation, develop a structure for recording data, consolidate INADs, identify pivotal study sites, streamline procedures, and share information.