

Second Annual Report of Progress

Performance Period: July 1, 1995 to June 30, 1996 (Year No. 2)

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

In 1994, the National Biological Service (NBS), U.S. Fish and Wildlife Service (FWS) and International Association of Fish and Wildlife Agencies (IAFWA), on behalf of the 50 states, developed an initiative to work cooperatively to fund and carry out the research required to gain approval of high priority drugs (therapeutants and anesthetics) and to demonstrate the concept of crop grouping. This unprecedented partnership is one of the largest and most important agreements ever forged on behalf of fish management, production, and disease control. The IAFWA Project began July 1, 1994 (Year 1) and presently is envisioned to extend until June 30, 1999 (Year 5). At its inception, 39 states agreed to contribute \$20,000 each to the initiative for five years for a total of \$ 3.9 million while NBS was to contribute \$867,000 per year for a total of \$4.3 million. In Year No. 2, 36 states contributed \$20,000 annually. Additionally, the NBS contribution was reduced from \$867,000 to \$767,000. In Year No. 3 (July 1, 1996 to June 30, 1997) it is anticipated that 37 states each will contribute \$20,000 to this effort. After an assessment of the remaining data requirements and the funding available through June 30, 1999, the IAFWA Project, as originally envisioned, has a shortfall of \$1.4 million and two years of time.

The specific objectives of the IAFWA Project are to develop data to 1) extend and expand existing New Animal Drug Applications (NADAs) for two high priority drugs, (2) gain NADA approvals for five therapeutants and one anesthetic/sedative to support public fish production in the United States, and (3) develop data to support the crop grouping concept. The IAFWA Project includes efforts to: 1) extend the NADA approval of **formalin** to additional fish species and their eggs; 2) expand the existing NADA approval for **oxytetracycline** to include other diseases and extend the label to include other species, 3) gain NADA approvals for all important public aquaculture species for use of **benzocaine, chloramine-T, copper sulfate, hydrogen peroxide, potassium permanganate, and sarafloxacin hydrochloride (sarafloxacin)**; and 4) develop research data to support the acceptance of a crop grouping concept by the Center for Veterinary Medicine (CVM). Based on the data generated, CVM will assess whether a few selected fish species can be used as surrogates for all or most of the cultured fishes in the United States.

After two years of intense activity, much progress has been made on many elements of the IAFWA Project. As expected with a project of this magnitude, certain situations and events have made it necessary to adjust the objectives, priorities, and sequence of work for the following drugs: 1) **Sarafloxacin** - redistribution and reprogramming of dollars and effort originally allotted to gain approval of sarafloxacin was necessary after the CVM announced that it would severely limit the use of fluoroquinolones in food animals; 2) **Hydrogen Peroxide** - substantial research effort was added to conduct regulated studies of hydrogen peroxide efficacy after a producer of hydrogen peroxide indicated that it would seek an NADA for aquaculture uses; 3) **Oxytetracycline** - substantial research effort was added when CVM requested that the existing residue method (microbial inhibition assay) for fish tissue on which the original approval for oxytetracycline was based, be replaced with an analytical regulatory method; 4) **Chloramine-T** - development of an assay for chloramine-T is being required by CVM to support analytical requirements to document chloramine-T concentrations in water of treatment facilities during pivotal efficacy studies; and 5) **Anesthetic/Sedative** - the efficacy and overall performance of *Aqui-S*, a newly identified anesthetic with a potential zero withdrawal time, is being evaluated relative to benzocaine before committing additional funds to gain the approval of benzocaine.

The purpose of this document is to report the progress and current status of each study element, identify expected products for the project, and anticipate project shortfalls. Changes in the IAFWA Project have produced some deviations from the original proposal; however, we remain optimistic that many of the objectives of the original IAFWA Project will be met. Highlights of the last performance year follow immediately and precede a detailed reporting of job elements within each study.

**HIGHLIGHTS: YEAR No. 2
(July 1, 1995 to June 30, 1996)**

- **Aqui-S (anesthetic/sedative):** In January 1996, the International Association of Fish and Wildlife Agencies (IAFWA) Project coordinators learned of a new anesthetic, Aqui-S, that had been approved in New Zealand with a zero withdrawal time. Because Aqui-S might be effective for use in public fish production in the United States, the Upper Mississippi Science Center (La Crosse, WI; UMSC) decided to evaluate and compare efficacy and regulatory requirements needed for approvals on Aqui-S and benzocaine.
- **Benzocaine (anesthetic/sedative):** A final report describing the effects of temperature on the loss of benzocaine and its major metabolite, acetylated benzocaine, from channel catfish fillet tissue was submitted to the Center for Veterinary Medicine's (CVM's) Office of Science. The study provides the agency with important information on the nature of benzocaine loss from treated fish and the relative composition of the residues in the edible portion of fish with time after exposure.
- **Chloramine-T (external antibacterial):** CVM accepted the data in two residue chemistry studies as satisfying requirements for total residue depletion and metabolism of chloramine-T in rainbow trout. They concluded from the data that para-toluene sulfonamide (p-TSA) is the major metabolite that results from chloramine-T exposure in fish. This information allowed the agency to declare p-TSA as the marker residue for chloramine-T in juvenile rainbow trout and to calculate a tolerance for chloramine-T in tissue of experimental fish.
- **Copper Sulfate (microbicide):** A residue chemistry study of copper sulfate was completed and submitted to CVM on April 4, 1996. Based on the data that indicated the lack of accumulation of copper residues in fish, CVM determined that there are no human food safety concerns over the use of copper sulfate as a therapeutic; thus, making approval of a New Animal Drug Application (NADA) relatively easy to obtain.
- **Formalin (microbicide):** CVM has accepted the data and conclusions of a target animal safety study on the toxicity of formalin to warm- and coolwater fish eggs that was submitted along with a proposed formalin label on December 15, 1995. This will allow for the eminent extension of the current formalin label as a drug to control and prevent saprolegniasis (fungal infections) on the eggs of Cypriniformes, Perciformes, Siluriformes (1,000 to 2,000 ppm) and Ascipenseriformes (concentrations < 1,500 ppm) for 15 minutes daily.
- **Hydrogen Peroxide (microbicide):** Hydrogen peroxide will retain its low regulatory priority (LRP) status; however, Eka Nobel Inc. initiated an investigational new animal drug (INAD) exemption process in January 1996 initially to control and prevent saprolegniasis on fish eggs. CVM stated in June of 1995 that LRP would not apply to external antibacterial or parasiticide uses; therefore, additional data requirements must be met for approvals in these two areas.
- **Hydrogen Peroxide (microbicide):** A target animal safety study on the toxicity of hydrogen peroxide to representative warm- and coolwater fish eggs was completed and the final report is in preparation. An additional report was submitted to CVM requesting an amendment to the current LRP ruling on hydrogen peroxide to allow its use at concentrations up to 1,000 ppm to control and prevent saprolegniasis on fish eggs. No decision has been received from CVM to date.

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- **Hydrogen Peroxide (microbicide):** A study to develop a method to uniformly and systematically induce saprolegniasis on channel catfish was completed. A protocol to develop a similar model for rainbow trout has also been written. These models will be used to produce infected fish for pivotal efficacy studies of hydrogen peroxide to control and prevent saprolegniasis. A pilot study to test the efficacy of hydrogen peroxide for treating saprolegniasis in channel catfish is underway.
- **Negotiations and Contract Coordination:** Based in part on progress made by the IAFWA Project, the Director of CVM announced at Aquaculture '96 that CVM anticipates near-term approvals for copper sulfate as a microbicide for all fish, formalin as a microbicide for all fish and fish eggs, and oxytetracycline as a marking agent for all fish. He listed several other project drugs for potential or anticipated NADA approvals by the year 2000: chloramine-T for control of bacterial gill disease and external flexibacteriosis for all fish, hydrogen peroxide as a fungicide for all fish, oxytetracycline as an antibacterial for shrimp and all fish, potassium permanganate as a microbicide for all fish, and sarafloxacin to control enteric septicemia in catfish.
- **Negotiations and Contract Coordination:** A program review of the IAFWA Project was conducted in May and June 1996 at UMSC to update and focus project objectives for the remaining three years of the project. Based on the assessment, an additional \$ 1.4 million and two years of time is required to fulfill the original IAFWA Project objectives.
- **Oxytetracycline (antibacterial, marking agent):** A preliminary report of the high performance liquid chromatography (HPLC) method for oxytetracycline in edible fish tissue was submitted to CVM and is being evaluated. Approval of the method by CVM will allow initiation of a bridging study for the HPLC method with the official microbial inhibition assay method. CVM is requiring a bridging study before residue depletion studies begin.
- **Potassium Permanganate (microbicide):** The exposure phase of a study to quantify the accumulation of manganese residues in edible muscle of channel catfish following exposure to waterborne potassium permanganate was initiated January 23, 1996 and exposures were completed on June 6, 1996. Sample analyses are underway and will be completed by November 1, 1996.
- **NOTE:** The Fish Farming Experimental Laboratory (FFEL) in Stuttgart, AR was officially transferred from the U.S. Department of the Interior to the Agricultural Research Service, U.S. Department of Agriculture. The UMSC will be transferred to the Biological Resources Division, U.S. Geological Survey, U.S. Department of the Interior on October 1, 1996.

SUMMARY OF PROGRESS BY RESEARCH STUDY PLAN

A summary follows on the progress made during the period from July 1, 1995 to June 30, 1996 (Year No. 2) 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 fungal infections on eggs and adults of publicly cultured freshwater fish.

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Expected Products: Approval of an amended NADA for formalin to control and prevent saprolegniasis (fungal infections) on all fish and fish eggs by the end of Year No. 3. Approval of an amended NADA for formalin to control external parasitic infestations on all fish by the end of Year No. 5.

Job No. 1 (NEW TITLE): Coordination of formalin compassionate Investigational New Animal Drug (INAD) exemptions and NADA submissions.

Progress: In August 1995, an INAD implementation and coordination meeting in Bozeman, MT was attended by the U.S. Fish and Wildlife Service (FWS) INAD coordinators, study monitors, the National NADA Coordinator, and the Upper Mississippi Science Center (UMSC) NADA coordinators. In November 1995, a meeting was held in Washington DC with the Center for Veterinary Medicine (CVM), the National NADA Coordinator, UMSC NADA coordinators, and most INAD coordinators to address the workload reduction plan announced by CVM and how to best consolidate all INADs.

UMSC staff continued to monitor the progress of the formalin compassionate INAD. Data from formalin treatments administered under the first year of the FWS INAD were gathered and discussed. The study protocol for the FWS formalin INAD was revised in November 1995 and approved for use by CVM for another year.

Efforts to identify hatcheries to conduct pivotal efficacy studies continue. UMSC has contacted INAD participants and discussed the eventual collection, pooling, and analysis of all data to support an amended NADA.

Current Status: The FWS formalin INAD has been revised and implemented for a second year.

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: A study to develop a method to uniformly and systematically induce saprolegniasis in channel catfish was completed. A protocol to develop a similar model for rainbow trout is in review.

Pivotal efficacy study protocols for treating saprolegniasis in fish are being developed that will use the models developed for channel catfish and rainbow trout.

A target animal safety study of formalin treatments on cool- and warmwater fish eggs has been completed and submitted to CVM (see Job No. 3 for details). Data from this study also contained information on the efficacy of formalin for controlling and preventing saprolegniasis.

Current Status: No further efficacy studies on fish eggs are planned at this time.

Research to develop a method to induce saprolegniasis in channel catfish is complete and studies to develop a similar model for rainbow trout will begin in September 1996. The protocols being developed to study the efficacy of hydrogen peroxide for treating saprolegniasis on channel catfish and rainbow trout will also be used to study the efficacy of formalin for treating saprolegniasis in channel catfish and rainbow trout when work with hydrogen peroxide is complete.

Job No. 3: 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.

Progress: CVM has accepted the data and conclusions of a target animal safety study on the safety of formalin to warm- and coolwater fish eggs that was submitted to CVM along with a

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proposed formalin label on December 15, 1995. These data will allow for the eminent extension of the current formalin label, under an amended NADA, to control and prevent saprolegniasis in the eggs of warmwater and other coolwater species of the orders Acipenseriformes (concentrations <1,500 ppm), Cypriniformes, Perciformes, and Siluriformes (concentrations from 1,000 to 2,000 ppm).

Current Status: The final report on the safety of formalin treatments on the eggs of cool- and warm-water fish was accepted by CVM and extension of the label to include the eggs of additional species of fish is being pursued. UMSC does not anticipate the need for target animal safety studies on fish at this time because CVM has accepted the concept of using a most sensitive representative species (striped bass) as a surrogate species to determine the safety of formalin. This was a direct result of studies conducted on striped bass by Auburn University (Auburn, AL) in support of a label extension for external parasitic infestations. UMSC will work to extend the concept of target animal safety data requirements for other waterborne drugs.

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.

Expected Products: Approval of an amended NADA for oxytetracycline as a marking agent on all fish in Year No. 3; approval of an amended NADA for oxytetracycline to control internal flexibacteriosis for all salmonids below and above 9°C by 2000; approval of an NADA for oxytetracycline to control internal flexibacteriosis in one representative cool- and one representative warmwater species by 2000.

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

Progress: UMSC personnel discussed progress to date and coordination of data acquisition from INADs for oxytetracycline with personnel from FWS and the Western Regional INAD Projects.

Review of data requirements to conduct pivotal efficacy studies identified the need for an analytical method in feed acceptable to CVM. Development of a protocol to address this need was initiated.

Current status: When the study on the analytical method in feed is completed in Year No. 3, the results will be forwarded to CVM for concurrence that the method is adequate for use in pivotal efficacy studies. UMSC will support pivotal studies by using this method to determine the concentration of oxytetracycline in feeds from participating hatcheries.

The oxytetracycline label can be expanded to other diseases in salmonids and catfish if pivotal efficacy data are supplied by INAD participants. To date, efficacy data on oxytetracycline for diseases other than internal flexibacteriosis have been collected only on a few occasions. This may jeopardize the development of pivotal efficacy data for these diseases.

Treatment of internal flexibacteriosis at higher dosages and for longer durations than the current label may result in additional data requirements such as marker residue depletion studies. No residue depletion studies can begin for any use until the dosage and duration used in pivotal efficacy studies is established (dosage in the protocols of some INADs was increased between the

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first and second treatment years). Because the efficacy data is important to the extension and expansion of the oxytetracycline NADA, UMSC will be working with INAD participants to identify pivotal study sites and develop the protocol for these studies.

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

Progress: Work on an analytical method using high performance liquid chromatography (HPLC) for oxytetracycline in edible fish tissue is under way to determine the accuracy and precision of the method for several species of fish (cold-, cool-, and warmwater). A preliminary report of the HPLC method was forwarded to CVM and is under evaluation. Determination that the method is acceptable by CVM will allow initiation of a bridging study for the HPLC method with the official microbial inhibition assay method.

CVM is requiring a bridging study before residue depletion studies can begin. A similar type of study was completed by the U.S. Food and Drug Administration (FDA) laboratories in Seattle, WA and Denver, CO for shrimp tissue. An additional bridging study with fish tissue was planned by the FDA laboratories; however, key personnel have left FDA. As a result, UMSC has initiated plans to conduct the bridging study in edible fish tissue. The UMSC has obtained documents from FDA on the bridging study for shrimp tissue, as a model, in order to conduct and coordinate the bridging study for fish tissue. UMSC will conduct the analytical portion of the bridging study and the FDA's laboratory in Denver will conduct the microbial inhibition assay.

Current status: Development of information on an HPLC method for oxytetracycline in fish will continue. A protocol will be developed to conduct a bridging study for the microbial inhibition assay and HPLC methods.

Residue depletion studies can begin when an acceptable bridging study of the HPLC method with the official microbial inhibition assay method is completed.

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

Progress: No activity during Year No. 2.

Current Status: The requirements for target animal safety are unclear at this time. Additional data requirements may result from the use of higher dosages for a longer duration in the treatment of internal flexibacteriosis.

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: Approval of an NADA for copper sulfate as a microbicide for all fish by Year No. 3.

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 protocol on the accumulation of copper residue in edible muscle of channel catfish following exposure to waterborne copper sulfate was approved by the National Center for Toxicological Research (NCTR) and CVM on September 30, 1994.

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Current Status: Job No. 1 completed.

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: A study on the accumulation of copper residues in edible tissue of channel catfish following exposure to waterborne copper sulfate was submitted to CVM and NCTR on April 4, 1996.

Current Status: Job No. 2 is considered complete. Based on the above report and similar research on tilapia by Tom Bell, CVM aquaculture specialist, CVM determined on July 11, 1996 that there are no human food safety concerns for use of copper sulfate as a therapeutic; thus, making an NADA approval relatively easy to obtain.

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

Progress: An EA on the effect of copper sulfate use in aquaculture is near completion and will be submitted to CVM by October 1, 1996. An environmental toxicologist is being consulted for final revisions and completion of fate and environmental effects sections of the final report.

Current Status: Based on preliminary analysis of the originally submitted EA, CVM determined that there are no environmental concerns for the use of copper sulfate as a therapeutic in aquaculture; thus, making an NADA approval relatively easy to obtain.

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: No activity in Year No. 2.

Current Status: Based on discussions with CVM, it is unlikely that residue data will be needed on any additional fish species.

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 external flexibacteriosis on cultured freshwater fish.

Expected Products: Approval of an NADA for chloramine-T to control and prevent BGD and external flexibacteriosis on salmonids and a representative species of cool- or warmwater fish by 2000.

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

Progress: With the ruling that the marker residue for chloramine-T is para-toluene sulfonamide (p-TSA), CVM has identified three required genotoxicity studies with p-TSA to support the approval of chloramine-T. UMSC sent a letter of request to CVM's Office of Science on June 14, 1996 to ask that they administer and monitor these studies. However, UMSC was informed by the NADA sponsor, Akzo Nobel Chemicals Inc. (Dobbs Ferry, NY), that some genotoxicity studies have been performed on p-TSA. Efforts are being made to obtain these studies.

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Current Status: Requests by UMSC in the June 14, 1996 letter to CVM's Office of Science have been withdrawn until the genotoxicity studies on p-TSA can be acquired and evaluated. It may be possible that no funds will need to be expended on any genotoxicity studies on p-TSA.

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

Progress: The CVM liaison to the National Research Support Project Number 7 (NRSP-7), Dr. Meg Oeller, was contacted concerning the requirements for an environmental assessment (EA) on chloramine-T and what options were available to the IAFWA Project for having the EA conducted. Dr. Oeller stated that NRSP-7 could perform the EA because chloramine-T is under an INAD with that program.

Current Status: UMSC will wait until the label claims for chloramine-T are delineated before requesting an EA through NRSP-7.

CVM must review environmental fate and effect data for chloramine-T before a decision is made whether environmental studies are required for the drug approval submission. Potential pharmaceutical sponsors may be able to supply much of the environmental fate information.

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

Progress: As part of the INAD process, two meetings of the INAD coordinators were held during this reporting period. The first INAD coordinators' meeting was convened at Bozeman, MT on August 1-4, 1995. There was also an INAD Coordinators Workshop held at Rockville, MD on November 1-2, 1995. Personnel from CVM attended this meeting and explained how to use the information gathered from the INAD process to support eventual NADA submissions.

The results from the first year of the FWS INAD for use of chloramine-T for treatment of BGD and external flexibacteriosis on fish raised at public aquaculture facilities were analyzed and discussed at both the Midcontinent Warmwater Fish Culture Workshop in Council Bluffs, IA and at a meeting of the executive committee of the Western Regional INAD in Corvallis, OR. Data collected during the first year were reviewed and used to revise the FWS INAD protocol for a second year of use. Lack of success in identifying sufficient and consistent instances of external flexibacteriosis at participating INAD facilities may jeopardize the IAFWA Project's attempts to add that disease to the label for chloramine-T because of the lack of available sites to conduct pivotal efficacy dose-titration studies and clinical field trials.

During a review of data requirements to conduct pivotal efficacy studies, UMSC personnel identified the need for an analytical method to detect chloramine-T concentrations in water that could be used at hatcheries participating as pivotal efficacy study sites. A protocol was drafted to conduct a bridging study to link an accepted HPLC method with other possible methods to analyze for chloramine-T in water. The developed methodology will support analytical requirements for the pivotal studies planned for chloramine-T and could be used in the future by hatchery personnel to document the concentrations of chloramine-T during a treatment. The protocol was submitted by UMSC staff to NRSP-7 as a proposal for funding in June 1996.

Protocols for pivotal efficacy studies are critical to support the label claims of chloramine-T to control BGD and external flexibacteriosis. Two types of studies are required, dose-titration studies and clinical field trials. Traditionally, dose-titration studies are conducted in controlled environments with replicated experimental units using restricted numbers of test animals. The studies are technically demanding because of the study design. Only selected hatcheries are

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expected to be able to accommodate the scientific demands of the protocols. Clinical field trials are less controlled and restrictive and may be conducted on typical hatchery facilities. The data submitted must be scientifically defensible in terms of study design, the statistical analysis of the data must be appropriate, and efficacy must be unambiguous for specific diseases. Studies conducted under these protocols at selected pivotal study sites will form the basis of the NADA submission that will support the use of chloramine-T to prevent and control BGD and external flexibacteriosis in cultured fish.

Current Status: Based on recommendations by UMSC, the protocols for the FWS chloramine-T INAD for Year No. 2 were revised to accommodate more effective treatment regimes and to include provisions for prophylactic treatments.

A bridging study between a simple analytical method for chloramine-T that can be used on hatcheries and an HPLC method will be conducted and a report submitted to CVM by January 1, 1997. If accepted by CVM, the simple method will be used to support the analytical requirements for documenting chloramine-T in water during pivotal study efficacy testing. If the method is found to be useful in defining chloramine-T concentrations in water, the technology may be transferred to production hatcheries where it could be used routinely to define concentrations during treatments.

UMSC staff are currently coordinating efforts to draft acceptable protocols to conduct pivotal efficacy studies that will be used to satisfy the efficacy label claims for chloramine-T as a therapeutic to control or prevent BGD and external flexibacteriosis on cultured fish.

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

Progress: CVM notified UMSC that two studies submitted to support the total residue depletion studies conducted with [¹⁴C]chloramine-T in exposed juvenile rainbow trout were adequate for the agency to calculate a tolerance for chloramine-T. Furthermore, CVM concluded that the major metabolite that results from chloramine-T treatment is p-TSA.

Current Status: With the rulings listed above, UMSC can begin to develop an analytical method for the marker residue of chloramine-T, p-TSA.

Job 5: Target animal safety studies in freshwater fish to support the approval of chloramine-T as a drug.

Progress: No activity in Year No. 2.

Current Status: In Year No. 3, UMSC will identify a sensitive representative cool- or warmwater species by acute toxicity testing. In Year No. 4, target animal safety studies will be conducted in both rainbow trout and a second species sensitive to chloramine-T.

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.

Expected Products: Approval of a NADA for sarafloxacin for control of enteric septicemia in catfish by 2000 based on NADA sponsor and NRSP-7 data submissions; extension and expansion

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of the expected label can only occur with an extension of the IAFWA Project for a minimum of two years.

Progress: This project has been given low priority since FDA has decided to severely restrict the development of all fluoroquinolones for food animals.

Current Status: In the fall of 1994, FDA placed a general moratorium on all non-human uses of fluoroquinolones. In August 1995, CVM approved the use of sarafloxacin in poultry under a veterinary prescription. Also at that time, CVM ruled that sarafloxacin could not be developed for use in aquaculture under a compassionate INAD. In February 1996, CVM announced its expected approval of sarafloxacin to control enteric septicemia (ESC) in channel catfish by 2000. The current sponsor (Abbott Laboratories) decided not to pursue the completion of the NADA for channel catfish as a business decision, but NRSP-7 agreed to assist in completing the NADA package.

When sarafloxacin gains approval to control ESC in channel catfish, it is possible that expansions and extensions of the label through an amended NADA could be gained for other diseases and fish species. Conditions placed on the use of sarafloxacin under an amended NADA would be: 1) extra-label use would be prohibited; 2) efficacy must be established for each disease for each species; 3) no efficacy data development would be possible under a compassionate INAD as currently exists for all of the other IAFWA drugs; 4) use of the drug would be only under the prescription of a veterinarian. While the conditions for use appear to be somewhat more restrictive, sarafloxacin is such a broad spectrum antibacterial that its availability to public fish production would be very advantageous.

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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.

Expected Products: Approval of an NADA for potassium permanganate as a microbicide for all fish by 2000.

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

Progress: A protocol on accumulation of manganese in edible muscle of channel catfish following exposure to waterborne potassium permanganate was prepared and submitted to NCTR and CVM for review. The protocol was approved by NCTR and CVM on September 17, 1995.

Current Status: Job No. 1 is complete.

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 exposure phase of the residue study on potassium permanganate was completed on June 6, 1996.

Current Status: Tissue analyses are underway to detect whether manganese residues accumulate in fish tissue. These studies should be completed by November 1, 1996.

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

Progress: A preliminary search for literature on the fate and effects of potassium permanganate has been completed and a reference file containing 400 entries has been compiled and is in review.

Current Status: The necessary environmental literature on potassium permanganate was collected and is in review.

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: Preliminary arrangements have been made with Dr. Randy MacMillan of Clear Springs Food Co., Buhl, ID, to perform exposures of rainbow trout to potassium permanganate and transfer samples from Clear Springs Food Co. to NCTR for residue analysis.

Current Status: A cooperative project with a private aquaculture concern is underway and could result in fulfillment of the residue chemistry data requirements for fish other than channel catfish.

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

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of freshwater fish. The original objectives of this work have been changed to reflect the original desires of public aquaculture to have an efficacious anesthetic with a zero withdrawal time.

Change In Status: In January 1996, IAFWA Project coordinators learned of a new anesthetic, Aqui-S, that is approved with a zero withdrawal time in New Zealand. Because Aqui-S might be effective for use in public fish production in the United States, UMSC decided to evaluate and compare efficacy and regulatory requirements needed for approval of Aqui-S and benzocaine.

Expected Products: A decision on whether to pursue an NADA for benzocaine or Aqui-S and identification of a sponsor for the candidate drug will be made in Year No. 3. Approval of an anesthetic/sedative is expected for all fish in Year No. 5.

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

Progress: Work on benzocaine was stopped in January 1996 when UMSC became aware of the new anesthetic, Aqui-S. Until a candidate anesthetic is selected, no additional effort will be made to seek a sponsor or conduct additional research on benzocaine.

Current Status: An evaluation of the efficacy, toxicity, and remaining regulatory requirements for Aqui-S is needed. A protocol is being prepared to determine the efficacy and toxicity of Aqui-S. This study will be completed in Year No. 3. After consulting with member states in the IAFWA Project and the FWS, a decision will be made whether to proceed with approval efforts for benzocaine or Aqui-S.

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

Progress: The final report from the study to define the effects of temperature on the loss of benzocaine and acetylated benzocaine in channel catfish fillet (Interagency Agreement Number 224-92-7036) was submitted to the CVM's Office of Science on October 5, 1995. Development of a method to extract, separate, and quantify benzocaine and its principle metabolite, acetylated benzocaine, in rainbow trout fillet was completed. A study was completed to define the effects of temperature on the loss of benzocaine and acetylated benzocaine in rainbow trout fillets and a report was drafted.

Current Status: The study to define the effects of temperature on the loss of benzocaine and acetylated benzocaine in channel catfish is complete. The analytical work for the companion study with rainbow trout is complete. A final report of the effect of temperature on the loss of benzocaine and acetylated benzocaine in rainbow trout fillet is in the final stages of internal review at UMSC and will be submitted to CVM early in Year No. 3.

The reports on the effect of temperature on the loss of benzocaine and acetylated benzocaine in channel catfish and rainbow trout will be submitted to CVM. If benzocaine is selected as the candidate anesthetic, a request will be made for a regulatory decision to determine the marker residue and the need for additional data.

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 in Year No. 2.

Current Status: Target animal safety studies will be initiated on a candidate anesthetic after similar studies are completed for chloramine-T and hydrogen peroxide.

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Job No. 4: Mutagenicity testing in support of the approval of benzocaine as an anesthetic/sedative.

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

Current Status: Job No. 4 is complete. No further activity is 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 U.S. public fish production.

Progress: CVM officials have indicated that only 90-day rodent and 90-day non-rodent feeding studies will be required. An interagency agreement with CVM has been executed that will allow Office of Science personnel to administer and monitor external contract studies related to benzocaine and other fishery chemicals. UMSC is withholding authority to initiate these studies until a decision is made on the status of benzocaine relative to Aqui-S.

Current Status: UMSC staff worked with staff of the CVM's Office of Science to draft and implement an interagency agreement to contract for these studies. No work will be initiated on these studies until a decision is made whether to pursue the approval of benzocaine or Aqui-S.

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 in Year No. 2.

Current Status: Environmental fate and effects information is not currently available for either Aqui-S or benzocaine but may be available from a pharmaceutical sponsor. Environmental fate and effect data requirements will be identified when a candidate anesthetic is selected.

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 regimens 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.

Expected Products: Approval of an NADA for hydrogen peroxide to control and prevent saprolegniasis on all fish and fish eggs, and completion of an assessment of its efficacy as an external antibacterial and parasiticide on fish by the end of Year No. 5.

Change in status: Hydrogen peroxide will retain its current LRP status to control and prevent saprolegniasis on fish and fish eggs; however, as a result of a request by Eka Nobel Inc. (Marietta, GA) in January 1996, an NADA for hydrogen peroxide will be 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 fungal infections of freshwater fish and fish eggs.

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Progress: Tests were performed to determine the efficacy of hydrogen peroxide to control and prevent saprolegniasis on Atlantic salmon eggs. Tests on several species of salmonid eggs have indicated that the most sensitive stage to hydrogen peroxide is the period when embryo formation begins (Day 5-10 for 12°C).

A study to compare the efficacy of hatchery egg incubation systems for delivering hydrogen peroxide treatments was completed. Egg jar incubators accurately delivered hydrogen peroxide treatment concentrations while Clark-Williamson and Heath Incubators diluted treatment concentrations.

A study to identify methods to systematically and uniformly induce saprolegniasis in channel catfish was completed. A protocol to develop a similar model for rainbow trout has also been written and is in review. These models will be used to produce infected fish for evaluating the efficacy of hydrogen peroxide to control and prevent saprolegniasis. A protocol for a pilot study to determine the efficacy of hydrogen peroxide for treating saprolegniasis in channel catfish has been approved and research is in progress.

Data from recently completed target animal safety studies also contained information on the efficacy of hydrogen peroxide for controlling saprolegniasis on the eggs of cool- and warmwater fish species (see Job No. 3).

Current Status: Hydrogen peroxide efficacy studies on eggs are complete and efficacy studies on fish will be conducted in Year No. 3. Hydrogen peroxide appears to be an effective drug to control saprolegniasis on fish and their 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: A study protocol to assess the efficacy and safety of hydrogen peroxide exposures to fish at hatcheries is in review. In Year No. 3, hydrogen peroxide efficacy studies will be conducted on diseased fish at selected federal and state hatcheries near UMSC.

Current Status: Studies to determine the efficacy of hydrogen peroxide to control external parasitic infestations and bacterial infections will begin in Year No. 3. Preliminary observations indicate that hydrogen peroxide may be effective in controlling external bacterial infections and parasitic infestations on freshwater fish.

Job No. 3: Conduct target animal safety studies on 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 channel catfish, common carp, lake sturgeon, northern pike, paddlefish, walleye, and white sucker were completed and a report is being prepared for an NADA submission to CVM. Previously, a report was submitted to CVM in support of a request to amend the current LRP status of hydrogen peroxide. This report requests an increase in the maximum allowable treatment concentration from 500 ppm to 1000 ppm for fish eggs with saprolegniasis. No response has been received from CVM to date.

A protocol to determine the safety of hydrogen peroxide on fish is in preparation.

Current Status: Report preparation of completed safety studies on eggs will continue in Year No. 3 for an NADA submission to CVM. Hydrogen peroxide appears to be safe for use on a wide variety of fish species and life stages. Data from these studies is being used to support

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amending the current LRP status of hydrogen peroxide to allow use of concentrations up to 1,000 ppm on fish eggs.

Data from acute toxicity studies on fish will be submitted to CVM to support the concept of using a most sensitive representative species as a surrogate species to satisfy target animal safety data requirements for an NADA approval of hydrogen peroxide.

STUDY NO. 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.

Expected Products: By Year No. 5, demonstration of crop grouping as a viable concept with CVM in developing residue chemistry data for aquaculture drugs, thus reducing future data requirements and associated costs.

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

Progress: Studies were initiated to adapt existing analytical methods for HPLC analysis of sarafloxacin to rainbow trout tissues. The study for the development of an analytical method in fish plasma and fillet tissue was initiated.

Current Status: Pharmacokinetic studies on sarafloxacin will begin after analytical methods are tested for accuracy and precision and found to be adequate.

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

Progress: No activity in Year No. 2.

Current Status: Work on this job will be initiated after more information on the pharmacokinetics and metabolism of sarafloxacin in rainbow trout is complete. Work in other diverse groups of fish will be initiated late in Year No. 3.

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

Progress: Work in this job focuses on (1) development and implementation of methods to collect reliable pharmacokinetic data from channel catfish using benzocaine as a waterborne test drug and (2) development of accurate and representative models of the accumulation, metabolism, and loss of benzocaine in fish. Initial work with channel catfish has been promising.

Methods to dose individual fish intravascularly with benzocaine and collect multiple serial blood samples from animals have been successful. Benzocaine loss from plasma of channel catfish most closely conformed to a three compartment model with half-lives of 6 min (\hat{A}), 46 min (), and 1240 min (). The clearance half-lives from the different compartments likely reflect the relative blood perfusion rates within the individual compartments with the lowest half-life representing highly perfused tissues and the highest half-life reflecting the slowly perfused tissues such as white

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muscle. The slowest half-life was considered to be the true biological half-life for benzocaine and is thought to reflect the large volume of distribution in the muscle mass rather than a low capacity for clearance from that compartment.

In another attempt to model benzocaine loss from catfish, the data collected from the compartmental analysis studies were fit to a simple physiologically-based pharmacokinetic model which has been developed for channel catfish with a different compound. The model for benzocaine in channel catfish is currently under development and is being refined to reflect the true nature of benzocaine loss from catfish. This type of model has been used successfully to conduct comparative pharmacokinetic studies of drugs or toxicants among mammalian species. If successful in fish, this approach would be extremely useful in validating a crop grouping concept for important aquaculture species.

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 in phylogenetically diverse species to support or refute a crop grouping concept for fish.

Progress: Work in this portion of the project is contingent upon the establishment and evaluation of reasonable compartmental models in channel catfish and rainbow trout. Much of the work being undertaken in the project at this time is to develop the model in channel catfish and rainbow trout. Loss patterns of benzocaine in other species will be evaluated with compartmental analyses and the information added to the data base as the reliability of the models in these species is established.

Current Status: This work is being deferred until a reasonable model for benzocaine pharmacokinetics is developed and validated in channel catfish (warmwater) and rainbow trout (coldwater). Subsequent work is likely to proceed rapidly once the methods are in place for these two surrogate species.

STUDY NO. 10: NEGOTIATIONS AND CONTRACT COORDINATION

Objectives: To 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. To coordinate the administration of all contracts by CVM's Office of Science to ensure efficiency, timeliness, and acceptability of data to CVM. To track and report the progress of all studies and ensure that they are proceeding for approval in a timely, logical, and efficient manner. To assemble and submit NADAs for approval by CVM.

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

Progress:

General -- The National NADA Coordinator and UMSC NADA coordinators held a meeting under CVM sponsorship on November 1-2, 1995 in Rockville, MD with INAD Coordinators. The objectives of the meeting were to: (1) increase communications between coordinators; (2) develop working relationships; (3) review the roles and responsibilities of INAD\NADA coordinators; (4) coordinate data generation for each drug; (5) consolidate INADs where possible; (6) determine the general format for INAD\NADA submissions; and (7) exchange information on current progress and any significant research findings for each drug. Twenty-two INAD\NADA coordinators and five CVM representatives participated in the workshop. The goals of the workshop were either met or plans were set in motion to accomplish the remainder.

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In addition, CVM presented their workload reduction plan at the workshop that will result in reduction of annual reviews and renewals.

A special session was held at the Midcontinent Warmwater Fish Culture Workshop in February 1996 in Council Bluffs, IA to consolidate and coordinate INADs, draft label claims, identify criteria for pivotal efficacy studies, and identify potential pivotal study sites for IAFWA drugs and other aquaculture drugs such as diquat and Cutrine Plus.

Several discussions were held between the National NADA Coordinator, UMSC staff, and CVM regarding CVM's aquaculture workload plan and the status and direction for chloramine-T, copper sulfate, formalin, hydrogen peroxide, and oxytetracycline. In addition, UMSC staff and the National NADA Coordinator have negotiated with CVM regarding label claims and regulatory requirements for pivotal efficacy study sites.

Several meetings were held at UMSC in May and June 1996 to review the whole IAFWA Project. Included in the discussions were the following topics on each study plan: (1) remaining data requirements; (2) tasks and jobs; (3) assignments for each job; (4) time table for completing each assigned task; (5) budget projections by study plan and year; (6) budget shortfalls for the original IAFWA Project; and (7) assessment of the potential products at the end of the IAFWA Project. Prior to these meetings, UMSC had already reprogrammed its effort and direction due to changes in requirements and circumstances for benzocaine, chloramine-T, hydrogen peroxide, oxytetracycline, and sarafloxacin. Efforts were made to save the entire IAFWA Project during government downsizing and budget reductions. Based on the assessment of the remaining data requirements and the funds available through June 30, 1999, the IAFWA Project, as originally envisioned, has a shortfall of \$1.4 million and two years of time.

Aqui-S -- The U.S. representative of Aqui-S was contacted and a meeting was held at UMSC on June 12, 1996 to discuss the potential for development of Aqui-S in the United States.

Benzocaine -- Discussions among the National NADA Coordinator, UMSC staff, and CVM personnel to obtain a sponsor for benzocaine resulted in a decision to contact prospective sponsors and invite them to consider sponsoring benzocaine NADAs. Each candidate will be asked what their contributions would be to the data package for an NADA. One potential sponsor of benzocaine has been contacted to discuss the possibility of developing an INAD/NADA. Additional work on benzocaine has stopped until a new anesthetic, Aqui-S, can be evaluated.

Chloramine-T -- UMSC representatives met with the current sponsor of chloramine-T, Akzo Nobel Chemicals, Inc., on August 16, 1995 to discuss the status of data development and procedures leading to an NADA. Recently, Akzo Nobel Chemicals Inc. made a commitment to provide the information necessary for the approval of chloramine-T in the United States as well as in Europe. On January 16, 1996, a meeting was also held with another potential sponsor of chloramine-T, H & S Chemical Company, Inc., concerning procedures for implementing an NADA.

Letters were sent through CVM to all holders of disclosed and undisclosed INADs of chloramine-T to announce that an effort to consolidate and coordinate these INADs, develop label claims, establish criteria for pivotal efficacy studies, and identify potential pivotal study sites, would be initiated at a meeting on INAD/NADAs in Council Bluffs, IA, on February 6-8, 1996.

Copper Sulfate -- CVM met the week of July 11, 1996 and determined that there are no human food or environmental safety concerns over the use of copper sulfate as a therapeutic, thus making approval relatively easy to obtain. A meeting was held with a potential sponsor of copper sulfate on July 15, 1996 to discuss the requirements for an NADA.

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Hydrogen Peroxide -- A meeting was held with representatives of a potential sponsor of an NADA on hydrogen peroxide, Eka Nobel, Inc. at UMSC on December 7, 1995 to discuss the data requirements, procedures, and status of data for a joint effort toward an NADA. As a result of these discussions, Eka Nobel Inc. submitted a INAD letter of intent (January 15, 1996) for hydrogen peroxide as a drug to control and prevent saprolegniasis and the company was notified on January 19, 1996 by CVM that they had been granted INAD No. 9671.

The IAFWA Project paid for the reformatting of an environmental report prepared for the approval of hydrogen peroxide in Canada and the report will be submitted into a veterinary master file to meet the environmental technical section requirements of an NADA.

Oxytetracycline -- CVM is near a decision on extending the NADA for use as a marking agent on species other than Pacific salmon; however, CVM has indicated that the INADs for the use of oxytetracycline for that purpose will continue until approval is granted.

Current Status: A meeting with CVM and a potential sponsor of copper sulfate will be held on August 5, 1996.

A meeting will be held in Kansas City, MO on November 7-8, 1996 to discuss the protocols and select the sites for pivotal efficacy studies on chloramine-T.

Progress has been made on interactions with current or potential sponsors of NADAs for Aqui-S, benzocaine, chloramine-T, copper sulfate, and hydrogen peroxide. Progress has been made on coordinating the INADs and determining how and where pivotal efficacy studies will be performed. Negotiations and discussions continue with CVM regarding data requirements for all IAFWA drugs.

Reprogramming for five of the eight drugs has occurred. Shortfalls of \$1.4 million and two years of time are envisioned for the IAFWA Project.

Job No. 2 (NEW TITLE): Coordinate the administration of contracts.

Progress: A memorandum of need was developed and an interagency agreement entitled "Drug toxicity studies for the support of drug approvals in aquatic animal species" was initiated on May 1, 1996 between CVM and UMSC to contract for required studies to support the approvals of the seven IAFWA Project drugs. The interagency agreement incurs \$200,000 and allows CVM's Office of Science to administer and monitor external contracts to support the approval of drugs for public fish production.

Specific projects identified within this project year include initiation of three genotoxicity studies for the primary degradation product of chloramine-T, p-TSA. UMSC sent a letter of request to CVM's Office of Science on June 14, 1996 to request that they administer and monitor three genotoxicity studies on p-TSA; however, UMSC was informed by the NADA sponsor, Akzo Nobel Chemicals Inc., that some genotoxicity studies have been performed on p-TSA. Efforts are being made to obtain the identified studies.

An interagency agreement was initiated on June 15, 1996 between NBS and the U.S. Department of Agriculture to partially fund the National NADA Coordinator position administered through Michigan State University during Year No. 3 of the IAFWA project.

A second research work order was developed and initiated with Dr. William L. Hayton, College of Pharmacy and Pharmaceutical Sciences, The Ohio State University, to undertake the benzocaine portion of the crop grouping study plan. The work will focus on the metabolism and pharmacokinetics of benzocaine in minor species such as sturgeon, yellow perch, and striped bass.

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Current Status: Three contracts were negotiated in Year No. 2 and UMSC anticipates these will continue in Year No. 3.

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

Progress: The following reports were written and distributed to all IAFWA project participants and stake holders: The first annual report of progress (July 1, 1994 to June 30, 1995); The second mid-year report of progress (July 1, 1995 to December 31, 1995); and the May 1996 progress update (January 1, 1996 to March 31, 1996).

Presentations were made on the IAFWA project to the FWS/INAD Coordination Workshop (Bozeman, MT, August 1995), U.S. Trout Farmers Association (Twin Falls, ID, September 1995), National Research Support Program Number 7 (Rockville, MD, October 1995), INAD/NADA Coordinators Workshop (Rockville, MD, November 1995), Midwest Fish and Wildlife Conference (Detroit, MI, December 1995), Coolwater Aquaculture Workshop (Kalamazoo, MI, January 1996), Midcontinent Warmwater Fish Culture Workshop (Council Bluffs, IA, February 1996), Western Regional Aquaculture Expo '96 (Sacramento, CA, February 1996), Aquaculture '96 (Arlington, TX, February 1996), Working Group on Quality Assurance in Aquaculture Production (Arlington, TX, February 1996), North Central Regional Aquaculture Center annual meeting (East Lansing, MI, February 1996), Great Lakes Fish Disease Workshop (La Crosse, WI, February 1996), Southeastern Fish Diagnosticians' Workshop (Mississippi State, MS, March 1996), International Association of Fish and Wildlife Agencies annual meeting (Tulsa, OK, March 1996), IAFWA ad hoc Committee on Aquaculture (Tulsa, OK, March 1996), Upper Mississippi River Research Consortium (La Crosse, WI, April 1996), Aquaculture Canada '96 (Ottawa, Canada, June 1996), Western Regional INAD Project Executive Committee (Portland, OR, June 1996) and the Western Fish Health Workshop (Corvallis, OR, June 1996).

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

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

Progress: Based in part on progress made by the IAFWA Project, the Director of CVM announced at Aquaculture '96 that CVM anticipates near-term approvals for copper sulfate as a microbicide for all fish, formalin as a microbicide for all fish and fish eggs, and oxytetracycline as a marking agent for all fish. He listed several other project drugs for potential or anticipated NADA approvals by 2000: chloramine-T for control of bacterial gill disease and flexibacteriosis for all fish, hydrogen peroxide as a fungicide for all fish, oxytetracycline as an antibacterial for shrimp and all fish, potassium permanganate as a microbicide for all fish, and sarafloxacin to control enteric septicemia in catfish.

During Year No. 2, IAFWA Project personnel submitted four NADA packages to CVM and received three responses as follows:

Benzocaine Submission. Effects of temperature on the loss of benzocaine and acetylated benzocaine residues from edible tissues of channel catfish (*Ictalurus punctatus*). October 5, 1995.

Chloramine -T, CVM response. CVM responded on July 14, 1995 to prior NADA submissions from UMSC by accepting data in two residue chemistry studies as satisfying requirements for total residue depletion and metabolism of chloramine-T in rainbow trout. They concluded from

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the data that p-TSA is the major metabolite that results from chloramine-T exposure in fish and that the agency has enough data to calculate a tolerance.

Copper Sulfate Submission. Accumulation of copper in edible muscle of channel catfish (*Ictalurus punctatus*) following exposure to copper sulfate. July 1, 1995.

Copper Sulfate, CVM response. CVM reviewed submitted data on residue chemistry and environmental safety and determined on July 11, 1996 that the agency has no human food or environmental safety concerns over the use of copper sulfate as a microbicide.

Formalin Submission. The safety of formalin treatments on fish eggs. December 15, 1995.

Formalin, CVM response. CVM stated on July 18, 1996 that formalin could be used safely on all fish eggs to control and prevent saprolegniasis if a statement was added to the label identifying the need for a preliminary bioassay on a subsample of fish before treatment of the entire group.

Hydrogen Peroxide Submission. The toxicity of hydrogen peroxide to representative warm- and coolwater fish eggs. February 29, 1996.

Current Status: UMSC and FFEL are pleased with the decisions by CVM concerning (1) the extension of the formalin NADA to control and prevent saprolegniasis on the eggs of all cultured freshwater fishes, thus eliminating the need for INADs for these uses; (2) the lack of concern over human food or environmental safety of copper sulfate making for a fairly easy NADA approval; (3) identity of p-TSA as the marker residue of chloramine-T and the possibility of establishing a tolerance. Both facilities also are encouraged by the announcement by the Director of CVM that he anticipates some form of NADA approvals for all the IAFWA drugs (with the exception of benzocaine) by 2000.

CVM will soon issue a notice in the Federal Register inviting sponsors to amend their formalin NADAs to include the extended claims for both the fungicide and parasiticide uses. These extensions of the formalin NADA to additional species will remove the need for INADs on formalin for these claims. The INADs on formalin's use to control and prevent saprolegniasis on fish will remain in effect.