

Third Midyear Report of Progress

Performance Period: July 1, 1996 to December 31, 1996

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.

The chronological midpoint of the IAFWA Project presents a good opportunity to reflect on the progress that has resulted over the last two and one-half years. Substantial progress toward approvals have been made in virtually every study area. Major successes in gaining approvals, such as formalin for the treatment of parasites on fish and for the treatment of saprolegniasis on eggs of all freshwater fish species, are perhaps the most representative examples of tangible achievements. However, additional successes have been realized by having certain critical studies accepted by CVM that will contribute to the approvals for copper sulfate, chloramine-T, oxytetracycline, hydrogen peroxide, and benzocaine.

In a larger sense, however, the more important, but intangible successes, for the IAFWA Project continue to be made on a daily basis. These successes are reflected in the cooperative and partnering interactions of the participants associated with the IAFWA Project. Participant interaction has taken on a variety of forms in the last two and one-half years. Most of the partnering and cooperation revolve around the development of efficacy assessments of therapeutants being evaluated within the project. Alliances between Federal, State and private aquaculture concerns have been forged on five of the eight therapeutants under investigation by the IAFWA Project. The U.S. Fish and Wildlife Service (FWS) established a national Investigational New Animal Drug (INAD) office in Bozeman, MT in 1994 that is coordinating all FWS-INAD related activities (e.g., protocol development, data collection and analysis). These activities involve 12 individual INAD exemptions, including 9 INADs for IAFWA Project therapeutants (one each for chloramine-T, copper sulfate, potassium permanganate, two for formalin, and 3 for oxytetracycline), 90 facilities, and approximately 300 INAD units. In November 1993, the Western Regional INAD Project was established as a government-private

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sector partnership to sponsor and manage clinical field trials for three therapeutants (oxytetracycline, chloramine-T, and formalin) at 218 facilities operated by six state conservation agencies, three tribal groups, and 24 private fish producers in Alaska, California, Idaho, Montana, Oregon and Washington. Twenty states have 61 individual INAD exemptions for eight therapeutants, one anesthetic, and three spawning aids. Many states have offered to consolidate their INADs with other states, and in some cases, with the private sector. More importantly, the groups are communicating, solving common problems, and sharing work-loads while working toward a common goal. Hopefully, this spirit of cooperation and involvement will continue after the initial project is completed.

The balance of this document reports on the progress and current status of each study element, identifies expected products for the project, and anticipates project shortfalls. As you read about the progress that has been made, reflect back on the spirit of cooperation that has made much of this progress possible. It is likely you will come to appreciate just how fully this project has benefitted public aquaculture in the last two and one-half years.

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Acronyms used in this report.

ADAA	Animal Drug Availability Act of 1996
BGD	Bacterial Gill Disease
BRD	Biological Resources Division, U.S. Geological Survey, U.S. Department of the Interior
CVM	Center for Veterinary Medicine, Food and Drug Administration, U.S. Department of Health and Human Services
EA	Environmental Assessment
ESC	Enteric Septicemia of Catfish
FDA	Food and Drug Administration, U.S. Department of Health and Human Services
FWS	Fish and Wildlife Service, U.S. Department of the Interior
GRAS	Generally Regarded As Safe
HPLC	High Performance Liquid Chromatography
INAD	Investigational New Animal Drug exemption
LRP	Low Regulatory Priority
NADA	New Animal Drug Application
NBS	National Biological Service, U.S. Department of the Interior
NCTR	National Center for Toxicological Research, Food and Drug Administration, U.S. Department of Health and Human Services
NRSP-7	National Research Support Program Number 7
PBPK	Physiologically Based Pharmacokinetic Model
PMF	Public Master File
p-TSA	para-toluene sulfonamide
SNARC	Stuttgart National Aquaculture Research Center, Agricultural Research Service, U.S. Department of Agriculture
UMSC	Upper Mississippi Science Center, Biological Resources Division, U.S. Geological Survey, U.S. Department of the Interior

HIGHLIGHTS:

(July 1, 1996 to December 31, 1996)

- **Copper Sulfate (microbicide):** A revised environmental assessment of copper sulfate was completed and submitted to CVM on December 20, 1996. Based on a previous review of the data that indicated the lack of hazard to the environment, CVM determined on July 11, 1996 that there are no environment safety concerns over the use of copper sulfate as a therapeutant, making approval of a New Animal Drug Application (NADA) relatively easy to obtain. A final evaluation and determination of environmental concerns by CVM is forthcoming.
- **Formalin (microbicide):** CVM placed a notice in the October 18, 1996 issue of the Federal Register inviting NADA sponsors of formalin to amend their labels to include the extended claims for both the fungicide uses (based on UMSC studies) and parasiticide uses (based on studies at Auburn University, Auburn, AL). When one formalin sponsor amends its NADA and label, formalin use will be extended to include treatments for the control and prevention of 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):** Studies to develop methods to uniformly and systematically induce saprolegniasis on channel catfish are complete and those for rainbow trout are nearly completed. Infected fish will be used in pivotal efficacy studies of hydrogen peroxide to control or prevent mortalities resulting from saprolegniasis. A pilot study to

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test the efficacy of hydrogen peroxide for treating saprolegniasis in channel catfish was conducted.

- **Negotiations and Contract Coordination:** A meeting was held with CVM on October 30, 1996 to clarify the design of protocols to conduct pivotal efficacy studies on chloramine-T. A chloramine-T pivotal study meeting was held in Kansas City, MO on November 7-8, 1996 with chloramine-T INAD coordinators to coordinate efforts on pivotal efficacy studies, finalize label claims, design protocols for pivotal clinical field trials, and identify pivotal study sites for chloramine-T. Commitments were obtained from several INAD coordinators to conduct these studies within the next year.
- **Oxytetracycline (microbiocide):** The tolerance for the amount of residues of tetracyclines (including oxytetracycline) in meats and seafoods was increased from 0.1 ppm to 2.0 ppm in edible muscle tissue by CVM on December 23, 1996 (21CFR Part 556). This new ruling should also reduce the withdrawal times for approved uses of oxytetracycline.
- **Oxytetracycline (microbiocide):** Data generated at the UMSC on the analytical method (HPLC) for oxytetracycline in the edible tissue of fish were reviewed by CVM and recommended as acceptable for use in a bridging study with the official microbial inhibition assay. The CVM has initiated steps to have the Denver FDA laboratory conduct the microbial inhibition assay portion of the bridging study. UMSC staff will coordinate the study and conduct the analytical phase.
- **NOTE:** The Fish Farming Experimental Laboratory in Stuttgart, AR, recently transferred from the U.S. Department of the Interior to the Agricultural Research Service, U.S. Department of Agriculture, has been renamed the Stuttgart National Aquaculture Research Center (SNARC). The UMSC was 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, 1996 to December 31, 1996 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.

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

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

Progress: Upper Mississippi Science Center (UMSC) staff continued to monitor the progress of six formalin compassionate INADs held by the states of Illinois, Pennsylvania, Texas, the Western Regional INAD Project, and the National Research Support Project 7 (NRSP-7). INAD

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coordinators have gathered efficacy data, discussed results with UMSC staff, and submitted annual reports to the Center for Veterinary Medicine (CVM). The study protocols for all six formalin INADs were revised and approved by CVM in 1996 for five additional years.

Current Status: Six formalin INADs were revised in 1996 and approved for five additional years. All INADs for prevention of saprolegniasis on fish eggs will terminate when the amended NADA for use of formalin to prevent saprolegniasis on all fish eggs becomes effective in 1997. The INADs for use of formalin to control saprolegniasis on fish will continue until efficacy data are accepted by CVM.

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: Studies to develop methods to uniformly and systematically induce saprolegniasis in channel catfish are complete and those for rainbow trout are nearly completed. Pivotal efficacy study protocols for treating saprolegniasis in fish are being developed for channel catfish and rainbow trout.

Current Status: The data on fish eggs are considered complete and no further studies are planned at this time.

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 (see Study No. 8).

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 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 along with a 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 (<1,500 ppm), Cypriniformes, Perciformes, and Siluriformes (1,000 to 2,000 ppm).

Current Status: All target animal safety data on formalin are considered complete and no further studies are planned at this time. Data to support the extension of the formalin NADA to control external protozoa and monogenetic trematodes on all finfish, and saprolegniasis on the eggs of all finfish were placed in Public Master File (PMF) 5228 and announced as accepted by CVM in the Federal Register (volume 61, number 203, pages 54445-54446). Sponsors need to submit amended or new NADAs to complete the approval process.

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.

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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 have kept abreast of the results of INADs for oxytetracycline from FWS and the Western Regional INAD projects. To date, the majority of treatments have been for flexibacteriosis: coldwater disease in coho salmon and columnaris in other salmonids. Treatments for columnaris have been at the upper end of the labeled dosage (3.75 g/100 lbs fish for 10 days) and treatments for coldwater disease have mostly been at dosages higher (7 to 12 g/100 lbs fish for 14 days) than allowed on the current label. The majority of treatments were defined as efficacious by a reduction in mortalities associated with flexibacteriosis.

In the coming year, the Western Regional INAD Project has requested (through CVM) that participants under their INAD compare the 10 g/14 day and 3.75 g/10 day use patterns for efficacy in side-by-side testing. These studies will be designed to determine if the higher dosage is more efficacious and will be important in determining if other data, such as residue depletion, will be required for dosages greater than the current label claims.

Jim Warren, Coordinator of the Western Regional INAD Project, met with CVM in September 1996 and discussed several items that will be important in conducting efficacy field trials for oxytetracycline. CVM suggested that data collected to date be analyzed to establish "historical controls" (i.e., multiple results of a single disease on a single fish species and hatchery). With the "historical control" information, treatments could be applied where approximately one-third would be placebos. Five requirements would have to be met for these studies to be initiated: (1) full cooperation and support of the participants, (2) standardized entrance requirements, (3) standardized success criteria for fish receiving medication in reference to those receiving the placebo, (4) procedures to determine when a placebo trial should be ended to prevent serious production losses, and (5) cooperation and support of the feed manufacturer before the start of the project.

Review of data requirements to conduct pivotal efficacy studies identified the need for an analytical method acceptable to CVM for quantifying oxytetracycline in feed. A protocol was developed by UMSC scientists to address this issue and the study under that protocol is near completion. Three types of feed were tested including feed types that covered the extremes in fat and protein content. Researchers at UMSC completed studies on the accuracy and precision for recovery of oxytetracycline spiked onto three types of unmedicated fish feeds. The analytical method was also tested on medicated feeds from manufacturers. Preliminary findings suggest that some feeds can have considerably different oxytetracycline content than listed on the label.

Current status: When studies on the analytical method for quantifying oxytetracycline in medicated feed and the storage stability of the samples are completed, results will be forwarded to CVM for concurrence that the method is acceptable. UMSC will use this method to quantify oxytetracycline in feed to support pivotal efficacy studies at hatcheries that agree to cooperate and coordinate their activities with the UMSC.

There is a urgent need for all holders of INADs for oxytetracycline medicated feed and UMSC to meet soon to decide on new label claims, develop protocols for pivotal efficacy studies, identify pivotal study sites, coordinate activities, and to support pivotal efficacy studies.

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Job No. 2 (NEW TITLE): Develop residue chemistry data on oxytetracycline in cold-, cool_, and warmwater fish.

Progress: Work is complete to determine the accuracy and precision of an analytical method for quantifying oxytetracycline in spiked edible tissue of rainbow trout, Atlantic salmon, walleye, lake sturgeon, and striped bass using high performance liquid chromatography (HPLC). A preliminary report of the method was forwarded to CVM for evaluation on June 17, 1996. Additional data including the complete chromatographic data for rainbow trout was also sent in response to questions by the reviewing CVM chemist on July 12, October 3, November 13, November 15, and December 5, 1996. The CVM chemist reviewing the method forwarded his recommendation to CVM on November 25, 1996 that the method was acceptable for use in a bridging study between the HPLC method and the official microbial inhibition assay method.

Current status: A protocol will be developed to conduct a bridging study between the official microbial inhibition assay and the HPLC method developed at the UMSC. The CVM has initiated steps for the Denver FDA lab to conduct the microbial inhibition assay portion of the bridging study. The UMSC will conduct the HPLC portion of the bridging study and use rainbow trout edible tissue since this species is most often tested in the oxytetracycline INADs and is considered a standard salmonid species for laboratory testing. The increase in the tolerance of the residue of oxytetracycline in edible fillet tissue of fish from 0.1 ppm to 2.0 ppm should also result in reduced withdrawal times.

Residue depletion studies will begin after 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 July 1, 1996 to December 31, 1996.

Current Status: The requirements for target animal safety are unclear at this time. Some studies have involved doses for marking that are higher than those allowed by the label (11.35 g/ 100 lbs for 4 days). If the higher dosages are found to be more efficacious in pivotal efficacy studies, additional data may be required. On the other hand, if the current marking label is extended by CVM to all freshwater fish, there may be no need to perform target animal safety studies for oxytetracycline.

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 therapeutic 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 prepared by personnel at the Stuttgart

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National Aquaculture Research Center (SNARC) and approved by the National Center for Toxicological Research (NCTR) and CVM on September 30, 1994.

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 report 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: A revised EA on the effect of copper sulfate use in aquaculture was completed and submitted to CVM on December 20, 1996.

Current Status: Based on preliminary analysis of the original 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. Pending a final review and a positive evaluation of the EA by CVM, Job No. 3 is considered complete.

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 July 1, 1996 to December 31, 1996.

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. The National NADA Coordinator has requested that Akzo Nobel

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Chemicals Inc., the NADA sponsor of chloramine-T, evaluate mutagenicity studies that had been previously generated on p-TSA.

Current Status: If the existing mutagenicity studies are adequate, it may be possible that no funds will be required for 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 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 is performing the EA because chloramine-T is under an INAD with that program.

Current Status: UMSC will wait until the EA has been evaluated by CVM before any requests are made to CVM concerning an opinion on any remaining data requirements.

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

Progress: Pivotal Efficacy Study Protocols

As part of the INAD/NADA process, two meetings were held during this reporting period. The first was convened in Rockville, MD on October 30, 1996 with CVM and several representatives of the International Association of Fish and Wildlife Agencies (IAFWA) Project, the U.S. Fish and Wildlife Service, and a state participating in the IAFWA Project (Pennsylvania). The group met with CVM personnel to discuss the design of the protocols for conducting pivotal efficacy studies on aquaculture drugs (especially chloramine-T) important to public fish production. This meeting was requested to facilitate the chloramine-T pivotal study meeting that was to be held with cooperators on November 7-8, 1996 in Kansas City, MO. The major conclusions reached as a result of the meeting with CVM are as follows: (1) no dose titration studies will be required if a definitive, effective low concentration for a drug has been identified through the compassionate investigational new animal drug (INAD) process; (2) label claims must make sense for field applications; (3) design of the label claims for chloramine-T to control or prevent external bacterial infections will probably be based on either increased survival or reduced mortalities, not on the reduction of the quantity of the disease pathogens or the intensity of the infections; (4) label claims drive the study design, therefore, of the three different categories of pivotal clinical field trial studies (preventive, control, and treatment), it is likely that only the first two categories of studies would be needed for chloramine-T or other water-borne drugs to determine the efficacy of the drug to control or prevent the mortalities associated with external bacterial or fungal infections; and (5) pivotal clinical field trial protocols could be designed to develop meaningful data at production hatcheries.

The second meeting was held in Kansas City, MO on November 7-8, 1996 to: (1) develop label claims for chloramine-T; (2) discuss the elements of the pivotal study protocols needed to support the label claims; and (3) obtain commitments to perform pivotal clinical field trials on chloramine-T. The aim of the pivotal study protocols will be to make the use of chloramine-T as broad as possible. The label claims will depend on the studies the group can perform. This workshop should provide the information needed by the investigators to design individual protocols that are suitable to the sites available to them.

After much discussion and reflection on the labels developed at the meeting in Council Bluffs, IA in February 1996, the group decided on the following label claims for chloramine-T:

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Flavobacterial Infections

For use on all freshwater fish in the families Ascipenseridae, Centrarchidae, Esocidae, Ictaluridae, Percichthyidae, Percidae, Polydontidae, and Salmonidae.

Indications: For the control or prevention of mortalities associated with external bacterial infections caused by flavobacters.

Directions for Use:

Control: Treat fish by immersion for 1 hr in a static bath or in a flow-through system for no more than one week per epizootic up to 4 times on consecutive or alternate days at concentrations from 10-20 mg/L.

Prevention: Treat only early life stage fish by immersion for 1 hr in a static bath or in a flow-through system no more than once per day up to 3 times per week at concentrations from 10-20 mg/L.

The important points gained from the meeting with CVM on October 30, 1996 were presented to the Workshop group regarding the design and major elements of pivotal study protocols. Computer diskettes were provided to the group that included (1) the protocols for the FWS compassionate INAD for chloramine-T (from Dr. Dave Erdahl, FWS), pivotal control study (from Jim Bowker, FWS), and pivotal prevention study (from Tom Cochran, PA), (2) the analytical method for chloramine-T (from UMSC), and (3) label claims (from UMSC). The group was also provided paper copies of the CVM document "Protocol development guideline for clinical effectiveness and target animal safety trials" as a guide to the format and elements considered by CVM to be important for pivotal study design. The suggestion was made to use a version of a compassionate chloramine-T INAD protocol as a template and add the elements needed for a pivotal study so that the investigator would not have to start from scratch.

The second day of the Workshop centered on (1) gaining commitments to conduct pivotal clinical field trials on chloramine-T, (2) determining how to coordinate development of an initial efficacy protocol that could be used as a template for others, and (3) consideration of the types of secondary supporting data that should be collected in concert with the pivotal studies. The group made several commitments to perform pivotal clinical field trials on chloramine-T. Those commitments are provided in the list available from UMSC entitled "Summary of potential facilities volunteering to conduct pivotal chloramine-T efficacy studies to prevent or control fish mortality resulting from external flavobacterial infections." The list includes the contact person/INAD sponsor, facility/participation confirmed (yes/no), species, time of year, size of fish (inches), and type of study (prevention/control).

Progress: Bridging Study to Support Chloramine-T Pivotal Efficacy Studies

UMSC identified a need to develop a simple, rapid, accurate analytical procedure to quantify chloramine-T in water that could be used in lieu of the currently accepted HPLC method. The method would be used on site by hatchery personnel to monitor chloramine-T concentrations in water during pivotal efficacy trials. The analytical method selected is used to determine total and free chlorine concentrations and by difference the concentration of bound chlorine which is converted to the concentration of chloramine-T. In a preliminary review of the more common methods for analysis of chlorine, a colorimetric method appeared to produce the sensitivity, accuracy, and precision necessary to fulfill the study objectives. Additional criteria that were considered while selecting the analytical method were ease of use, analysis time, and cost. A test kit based on colorimetric determination of chlorine was selected for initial evaluation and is commercially available from the Hach Company (Loveland, CO).

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Current Status: Dr. Dave Erdahl and Jim Bowker offered to host the next meeting of the pivotal study group in conjunction with their annual FWS-INAD Workshop. The meetings are scheduled for August 6-8, 1997 in Bozeman, MT. Several pivotal efficacy studies should have been performed by August 1997 so UMSC should have a clear idea where future efforts will need to be focused to conduct pivotal efficacy studies that will be used to satisfy the efficacy label claims for chloramine-T as a therapeutant to control or prevent mortalities associated with BGD and external flexibacteriosis on cultured fish.

Research is underway to evaluate the performance of the Hach test kit to determine chloramine-T concentrations in a variety of water conditions that might be encountered in public aquaculture. The performance of the test kit under a variety of water quality conditions remains to be evaluated including selected ranges of pH, temperature, hardness, organic content/chlorine demand, and fish load densities. A final report comparing the two methods will be submitted to CVM in early February 1997. UMSC is optimistic that this new method will be acceptable to CVM as an alternative to quantification of chloramine-T by HPLC. When this method has been validated against the accepted HPLC method, it will allow fish culturists to routinely monitor chloramine-T treatment concentrations to assure that the treated fish have received the correct concentration. When the method has been accepted by CVM, UMSC will provide test kits and training materials for the method to each participating hatchery.

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

Progress: No activity July 1, 1996 to December 31, 1996.

Current Status: Progress in this work unit has been delayed by lack of available personnel to work on an acceptable regulatory/confirmatory method for p-TSA. When work is complete on the study to bridge the colorimetric method for chlorine with the HPLC method accepted by CVM (see project in Job No. 3 above), work will progress on development of an analytical method that will satisfy the marker residue requirements for regulatory and confirmatory identification.

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

Progress: No activity July 1, 1996 to December 31, 1996.

Current Status: No progress has been made on this job because of the high priority need to develop a bridging method to support work in Job No. 3 above. After work on target animal safety studies are completed for hydrogen peroxide (see Study No. 8), UMSC will select representative cool- or warmwater species and initiate target animal safety studies for both rainbow trout and a second cool- or warmwater 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 to control 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 (ESC) by 2000 based on NADA sponsor and NRSP-7 data submissions; extension and

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

Progress: Development of data on sarafloxacin has been given low priority since FDA decided to severely restrict the development of all fluoroquinolones for food animals. On October 30, 1996, the National NADA Coordinator and Dr. William Gingerich met with Dr. Meg Oeller, CVM liaison to NRSP-7, to determine the status of sarafloxacin within CVM, NRSP-7, and the Centers for Disease Control. When the current sponsor (Abbott Laboratories) decided not to pursue the completion of the NADA for channel catfish as a business decision, NRSP-7 agreed to assist in completing the NADA package; however, NRSP-7 needs Abbott Laboratories to request assistance from NRSP-7. Once this request is received, the remaining requirements will take from eight to twelve months to complete.

Current Status: NRSP-7 will work to complete the requirements on sarafloxacin to control ESC. When sarafloxacin is approved, 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 aquaculture drugs including IAFWA Project drugs; and (4) use of the drug would be possible 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.

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 prepared by SNARC personnel on accumulation of manganese in edible muscle of channel catfish following exposure to waterborne potassium permanganate was 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 by SNARC personnel on June 6, 1996. Tissue analyses to quantify manganese residues in fish tissues is nearly complete. Preliminary data suggests that the concentrations of manganese in edible tissues are not affected by the potassium permanganate exposures employed in the study.

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Current Status: Tissue analyses to quantify manganese residues in fish tissue after exposure to potassium permanganate should be completed early in 1997.

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

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

Current Status: The literature review on the fate and effects of potassium permanganate will be prepared and used to support an EA.

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 by SNARC staff with Dr. Randy MacMillan of Clear Springs Food Co. (Buhl, ID) to expose rainbow trout to potassium permanganate and transfer samples from Clear Springs Food Co. to SNARC for residue analysis. Sample preparation for manganese analysis has begun. Quality assurance practices were developed at NCTR and implemented at SNARC to insure proper documentation of chain of custody when samples are transferred for final 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. SNARC expects to receive samples for manganese analysis by March 1, 1997.

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

Objectives (REVISED OBJECTIVES): To develop efficacy, target animal safety, and residue depletion data required for the approval of benzocaine/Aqui-S as an anesthetic/sedative with a short withdrawal time for several species 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 a NADA for benzocaine or Aqui_S and identification of a sponsor for benzocaine, if it is the selected anesthetic/sedative, will be made in Year No. 3. Approval of Aqui-S as the candidate anesthetic/sedative may be possible for at least one species of fish in Year No. 5.

Job No. 1 (REVISED TITLE): Develop a compassionate INAD request to evaluate benzocaine/Aqui-S as an anesthetic/sedative for fish cultured on public hatcheries.

Progress: Work to gain approval 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.

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A protocol was prepared by UMSC staff and preliminary data on efficacy and toxicity of AQUI-S were collected.

Current Status: Efficacy and toxicity studies of AQUI-S on several fish species in two size classes will be completed in Year No. 3. A determination will also be made of remaining regulatory requirements for AQUI-S. 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.

AQUI-S contains an ingredient that is similar to a major component in clove oil that also has anesthetic qualities in fish. The National NADA Coordinator recently learned that CVM has no intention of granting LRP status to clove oil, even though it has a Generally Recognized As Safe (GRAS) designation as a food additive. Approval of clove oil would require data on efficacy and target animal safety at a minimum, and, unlike AQUI-S, clove oil does not have a sponsor.

Job No. 2 (REVISED TITLE): Conduct residue chemistry studies in freshwater fish to support the use of benzocaine/AQUI-S.

Progress: The final report from the study to define the effects of temperature on the loss of benzocaine and acetylated benzocaine in rainbow trout fillet was archived at UMSC.

Current Status: Submission of a report on the effect of temperature on the loss of benzocaine and acetylated benzocaine in rainbow trout to CVM has been delayed until a decision has been made on which anesthetic will be pursued under the IAFWA Project. If benzocaine is selected as the candidate anesthetic, the rainbow trout residue report will be submitted with a request for a regulatory decision from CVM to determine the marker residue and the need for additional data.

No effort has been expended by the UMSC to determine the residue chemistry requirements for AQUI-S; however, the sponsor of AQUI-S is seeking to identify a contractor to perform a total residue depletion and metabolism study.

Job No. 3 (REVISED TITLE): Conduct target animal safety studies with benzocaine/AQUI-S in rainbow trout and a second species (cool_ or warmwater).

Progress: No activity July 1, 1996 to December 31, 1996.

Current Status: Target animal safety studies will be initiated when a candidate anesthetic is selected and after similar studies are completed for chloramine_T and hydrogen peroxide.

Preliminary information on the toxicity of AQUI-S is being developed for several fish species.

Job No. 4 (REVISED TITLE): Coordinate mutagenicity testing in support of the approval of benzocaine/AQUI-S.

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: All mutagenicity testing for benzocaine is considered complete and no further studies are planned at this time.

Data requirements on mutagenicity for AQUI-S are not known at this time.

Job No. 5 (REVISED TITLE): Coordinate subacute mammalian toxicity studies to support the approval of benzocaine/AQUI-S.

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Progress: CVM officials have indicated that only 90_day rodent and 90_day non_rodent feeding studies will be required for benzocaine to complete this data requirement. 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 to pursue an approval of benzocaine or Aqúi_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 Aqúi_S.

Job No. 6 (REVISED TITLE): Prepare an environmental assessment of the fate and effects of the release of benzocaine/Aqúi-S.

Progress: No activity July 1, 1996 to December 31, 1996.

Current Status: Environmental fate and effects information is not currently available for either Aqúi_S or benzocaine but may be available from a pharmaceutical sponsor or NRSP-7 in the form of an EA. 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 mortalities associated with 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.

Progress: Studies to identify methods to systematically and uniformly induce saprolegniasis in channel catfish are complete and those for rainbow trout are nearly complete. Infection models will be used to produce fish with saprolegniasis for evaluating the efficacy of hydrogen peroxide to control and prevent mortalities associated with saprolegniasis. A pilot study to determine the efficacy of hydrogen peroxide for treating saprolegniasis in channel catfish was conducted.

The final report on a study to determine the safety of hydrogen peroxide for treating saprolegniasis on the eggs of eight cool- and warmwater fish species is in review at the UMSC and will be submitted to CVM in Year No. 3 for inclusion in the hydrogen peroxide public master

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file (PMF). This study also contains information on the efficacy of hydrogen peroxide for treating saprolegniasis on fish eggs.

A study comparing the efficacy of several hatchery egg incubation systems to deliver hydrogen peroxide treatments was completed. Results indicate that expected treatment concentrations were often not realized throughout different egg incubation systems. Mc Donald egg jar incubators accurately delivered treatment concentrations whereas treatment concentrations delivered in Clark-Williamson and Heath Incubators were diluted. Researchers and fish culturists should periodically verify the accuracy of treatment dosing methods to ensure that expected concentrations are met.

Current Status: Hydrogen peroxide efficacy studies on eggs are complete and efficacy studies on fish will continue in the second half of Year No. 3. Hydrogen peroxide appears to be an effective drug to control mortalities associated with 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 control of external parasitic infestations and mortalities associated with external bacterial infections by hydrogen peroxide treatment has been drafted and is in review by UMSC staff. In Year No. 3, pivotal dose titration studies testing three concentrations of hydrogen peroxide will be conducted on fish infected with external bacteria or infested with external parasites at hatcheries near UMSC.

Current Status: Pivotal dose titration studies to determine the efficacy of hydrogen peroxide to control external parasitic infestations and the mortalities associated with external bacterial infections will begin in Year No. 3. Preliminary observations indicate that hydrogen peroxide may control mortality associated with external bacterial infections and control external 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 therapeutant 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. Previously, on February 29, 1996, 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.

Target animal safety studies on the eggs of rainbow trout were completed. Based on the results of this study, rainbow trout eggs may be safely treated with hydrogen peroxide at ≤ 1000 uL/L. The most sensitive egg stage to hydrogen peroxide treatment is blastopore formation through closure (Day 6-8 at 12 C) for rainbow trout. Steelhead rainbow trout eggs were more sensitive to hydrogen peroxide than non-steelhead rainbow trout eggs, especially those eggs from a strain with a history of Early Mortality Syndrome.

Current Status: Target animal safety data on hydrogen peroxide for cool- and warmwater fish eggs are complete and a report is being prepared to submit in Year No. 3 to CVM for inclusion into the hydrogen peroxide PMF. Data from these studies are also being used to support amending the current LRP status of hydrogen peroxide to allow use of concentrations up to 1,000 ppm on most fish eggs. Based on the data gathered in rainbow trout egg target animal safety

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studies, UMSC does not recommend treatment of steelhead rainbow trout eggs above 500 uL/L during the sensitive stage (blastopore formation through closure).

A protocol to determine the most sensitive representative species of fish to evaluate the safety of hydrogen peroxide to fish is in review by UMSC staff. 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 for other 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 demonstrate to CVM that crop grouping is a viable concept 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. An HPLC method for analysis of sarafloxacin in the plasma of rainbow trout and channel catfish has been refined and the accuracy and precision determined.

Similar studies for an analytical method for sarafloxacin in the edible fillet of channel catfish and rainbow trout have been initiated.

Current Status: A protocol to study the pharmacokinetics of sarafloxacin in channel catfish and rainbow trout is in review and should be initiated in Year 3.

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

Progress: No activity July 1, 1996 to December 31, 1996.

Current Status: Work on this job will be initiated after development of information on the pharmacokinetics and metabolism of sarafloxacin in rainbow trout and channel catfish. Work should be initiated in Year No. 4.

Job No. 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 and rainbow trout 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. To establish the details of benzocaine disposition, initial work has utilized the intraarterial administration of benzocaine.

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Benzocaine pharmacokinetics and metabolism were characterized in channel catfish as a part of the effort toward approval of benzocaine for use as an anesthetic for fish. Catfish at 21°C were administered benzocaine (1, 10, and 50 mg/kg) intraarterially over 30 minutes at a constant rate of infusion. Blood samples were collected over 48 hours and the plasma concentrations of benzocaine and its metabolites were determined by HPLC. Benzocaine, acetylated benzocaine, p-amino benzoic acid, and acetylated p-amino benzoic acid were observed, but there was no evidence of p-amino hippuric acid as is seen in mammalian species. At 48 hours, the fish were sacrificed, and several tissues were removed for analysis of benzocaine and its metabolites. The benzocaine plasma-time profile appeared to be triexponential and a three compartment model (WinNonlin software) was used to characterize its pharmacokinetics. The relatively long biological half-life (about 20 hours) was due to a large volume of distribution rather than the total body clearance, which was roughly equal to the cardiac output. A Physiologically-Based Pharmacokinetic (PBPK) model (ACSL software) was also developed that included six distributive tissue groups (gills, slowly perfused, rapidly perfused, fat, liver, and kidney), and three elimination and/or metabolism tissue groups (liver, kidney, and gill).

In another attempt to model benzocaine loss from catfish, the data collected from the compartmental analysis studies were compared with the prediction of a simple PBPK model using physiological parameters determined for channel catfish by other investigators. The model for benzocaine in channel catfish was refined since its original testing in early July 1996. Simulations were run to determine the goodness of fit of the model to analytical data collected in these studies from plasma, kidney and muscle tissues. These profiles agree with the experimentally determined concentrations observed after intraarterial administration of benzocaine to catfish, particularly for the plasma. Refinement of this model is underway. This model will permit examination of the behavior of benzocaine in fish of different size and at different temperatures and will help to identify the physiological and biochemical characteristics of fish that influence the pharmacokinetics and metabolism of benzocaine. Initial simulations indicate that temperature-induced changes in cardiac output have major impact on tissue concentration-time profiles. Other physiological parameters may prove important; only cardiac output has been examined thus far.

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 No. 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 and PBPK 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. Work for refinement of the model being developed for channel catfish and reported in Job No. 3 above was applied to diverse species including sturgeon, catfish, carp, and trout. Initial simulations indicate that both cardiac output and gill surface areas of different species provide evidence for substantial species differences in clearance of benzocaine. With a more refined model, the impact of interspecific differences in blood flows, tissue volumes, elimination clearances, tissue-to-plasma distribution coefficients and differences in metabolism can be quantitatively assessed. This should demonstrate that benzocaine pharmacokinetics are similar among diverse species and should permit rational identification of "crop groups".

Current Status: Development of the PBPK model for benzocaine pharmacokinetics is continuing in channel catfish (warmwater) and rainbow trout (coldwater). Physiological parameters for minor species are being collected. Along with the model development work, laboratory studies in white sturgeon, striped bass, and walleye will be used to validate and refine

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the model. Subsequent work is likely to proceed rapidly once the methods are in place for channel catfish and rainbow trout.

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 toward 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

Several discussions were held between the National NADA Coordinator, UMSC staff, SNARC staff and CVM regarding 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.

Three recent events will have an impact on the approval process and data requirements for IAFWA Project drugs. The Animal Drug Availability Act of 1996 (ADAA), signed into law on October 9, 1996, provides clearer definition of the "substantial evidence" required for NADA approvals, streamlines the consideration process by CVM, establishes a new drug category of a "veterinary feed additive", and simplifies licensing procedures for feed mills. These changes in the Food, Drug, and Cosmetic Act should help the IAFWA Project gain approvals for its priority drugs. The IAFWA Project will work with CVM for any additional clarification of ADAA rulings.

CVM released its new draft "A guide to the approval of animal drugs for minor uses and for minor species" in September 1996. This guide provides suggested protocols for drug approvals for multiple species at one time, simplifies requirements for approvals, and increases flexibility.

CVM held a Joint Canadian-United States Workshop on Jurisdiction of Sea Lice Treatment and Control in September 1996 that will impact IAFWA Project drug approvals. One of the action items resulting from the Workshop is the strategies and mechanics to institute forums for harmonization activities, i.e., the establishment of a joint Canada and United States Aquaculture Working Group. This means that data could be shared and certain requirements for all drugs could be harmonized so that there could be joint submissions leading to approvals being granted simultaneously in both countries.

Progress: Specific Drugs

Aqui-S -- Jan Holland, representative for the NADA sponsor of Aqui-S (Crop & Food Research, Nelson, New Zealand) met with the National NADA Coordinator and UMSC staff on August 20, 1996 in La Crosse, WI to discuss the research developed on Aqui-S in New Zealand, efficacious range of concentrations for various aquatic species, and potential for development of Aqui-S in the United States under the IAFWA Project.

The National NADA Coordinator contacted CVM to determine whether oil of cloves could be considered under a LRP status. CVM responded on December 6, 1996 that the agency will not

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consider clove oil to be a LRP drug when used as a fish anesthetic. Therefore, sponsors interested in fish anesthetic/sedative INADs/NADAs can continue to pursue them without concern for competition from unapproved clove oil products.

Benzocaine -- Nine potential sponsors of benzocaine were sent letters on September 4, 1996 requesting an indication of continued interest in being kept informed on benzocaine or becoming an NADA sponsor of benzocaine. Two pharmaceutical firms expressed an interest in becoming sponsors if the IAFWA Project selects benzocaine as the candidate anesthetic.

Chloramine-T -- The National NADA Coordinator, INAD coordinators, and a representative of UMSC met with CVM on October 30, 1996 to clarify the design of protocols to conduct pivotal efficacy studies on chloramine-T. A chloramine-T pivotal study meeting was held in Kansas City, MO on November 7-8, 1996 with INAD coordinators to coordinate efforts on pivotal efficacy studies, finalize label claims, design protocols for pivotal clinical field trials, and identify pivotal study sites for chloramine-T. Commitments were obtained from several INAD coordinators to conduct these studies within the next year.

Copper Sulfate -- CVM met July 11, 1996 and determined that there are no human food or environmental safety concerns over the use of copper sulfate as an therapeutant, thus making its approval relatively easy to obtain. A meeting was held July 15, 1996 in Memphis, TN with a potential NADA sponsor, Phelps Dodge Refining Corporation (El Paso, TX), to discuss their interest in sponsoring an INAD/NADA on copper sulfate, data requirements for approval, and coordination activities. A follow-up meeting with CVM and Phelps Dodge Refining Corporation was held on August 5, 1996 to develop an action plan needed to obtain approval of copper sulfate as a microbicide.

An environmental assessment on the effect of copper sulfate use in aquaculture has been completed by the SNARC and an outside consultant. The document was submitted to CVM on December 20, 1996.

Formalin -- CVM placed a notice in the October 18, 1996 issue of the Federal Register inviting NADA sponsors of formalin to amend their labels to include the extended claims for both the fungicide use on eggs (based on UMSC studies) and parasiticide use (based on studies at Auburn University, Auburn, AL). These extensions of the formalin NADA to additional species will remove the need for INADs on formalin for these claims. On October 28, 1996, the NADA Coordinator sent letters to the three current NADA sponsors of formalin inviting them to amend their NADAs and change their labels to reflect the extended claims granted by CVM. The INADs on formalin's use as a fungicide on fish will remain in effect until data are submitted to cover this use.

Hydrogen Peroxide -- The National NADA Coordinator and UMSC staff sent a letter and supporting documentation on August 27, 1996 to CVM summarizing the preliminary data and information on the safety and efficacy of hydrogen peroxide. Hydrogen peroxide has potential to be safe and effective at exposures up to 250 L/L for 15 to 45 minutes to control mortalities associated with external bacterial infections and to control external parasitic infestations on certain species of freshwater fish. This treatment regimen is well below the LRP concentration of 500 L/L allowed for the control of mortalities associated with saprolegniasis on all fish species and life stages.

Detailed information was provided to the NADA sponsor (Eka Nobel Chemicals Inc., Marietta, GA) on the status of hydrogen peroxide under the IAFWA Project.

Oxytetracycline -- The National NADA Coordinator and UMSC staff continued to coordinate the activities related to oxytetracycline as an antibacterial through the IAFWA Project, especially

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concerning the development of pivotal efficacy data. They have interacted with Jim Warren (Western Regional INAD Project, Portland, OR) regarding his discussions with CVM on September 19, 1996 concerning the use of voluminous efficacy data as "historical controls" and placebo feeds to gain early extension and expansion of the current oxytetracycline label.

A private fish producer was helped in their efforts to gain access to the oxytetracycline INAD held by the State of Texas. The National NADA Coordinator has reviewed the protocol being developed for control of strep infections in tilapia and largemouth bass.

Sarafloxacin -- The National NADA Coordinator and Dr. William Gingerich (UMSC) met with the CVM liaison to NRSP-7 on October 30, 1996 to discuss ways to complete the approval process for sarafloxacin to control ESC. The NADA for sarafloxacin is held by Abbott Laboratories (North Chicago, IL).

Current Status: Progress has been made on interactions with current or potential sponsors of NADAs for Aqu-i-S, benzocaine, chloramine-T, copper sulfate, formalin, 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.

Job No. 2: 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. This agreement encumbers \$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. This contract was extended to September 30, 1997 to allow for identification and implementation of external contracts that may be required for the approval of the therapeutants/anesthetics under study.

A cooperative research and development agreement is being developed between UMSC and the New Zealand Institute for Crop and Food Research Limited to cover cooperative efforts on Aqu-i-S.

Current Status: Three contracts were negotiated in Year No. 2 and UMSC anticipates these will continue in Year No. 3. One contract is being negotiated in this reporting period.

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

Progress: The following report was written and distributed in this reporting period to all IAFWA Project participants and stake holders: The second annual report of progress (July 1, 1995 to June 30, 1996). Products (NADA submissions, study protocols, publications, special reports, and presentations) that are a part of the IAFWA Project are reported to December 31, 1996 in Appendix I. In the future, the products will first be reported in Job No. 3 and then placed in the accumulative Appendix.

Major advances were made toward communication and coordination of INAD/NADAs of high priority drugs important to public fish production at a workshop held by the FWS in Bozeman, MT on August 14-15, 1996. Discussions centered on developing the pivotal data needed to complete the efficacy portion of the NADAs.

The Working Group on Quality Assurance in Aquaculture Production met on September 20, 1996 in Washington, DC and developed a five-year strategic plan that includes the IAFWA Project drugs.

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The National NADA Coordinator and Dr. William Gingerich (UMSC) met on October 30, 1996 in Rockville, MD with Dr. Meg Oeller, CVM Liaison to NRSP-7, to discuss coordination of the mutual projects that NRSP-7 and the IAFWA Project have in common. Both projects are working on chloramine-T, copper sulfate, hydrogen peroxide oxytetracycline, potassium permanganate, and sarafloxacin.

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

The IAFWA Project will be featured at a special session entitled "Partnerships for aquaculture drug approvals: models for success" to be held at World Aquaculture '97, Seattle, WA, February 19-23, 1997.

A Workshop on International Harmonization for Aquaculture Drugs and Biologics is scheduled to be held in Seattle, WA on February 24, 1997 to create an educational forum to exchange information and identify issues among public and private sectors and international organizations with the goal of initiating follow-up strategies to advance harmonization of drug maximum residue levels, aquaculture drug approval standards and biologics licensure.

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

Progress: From July 1, 1996 to December 31, 1996, IAFWA Project personnel submitted two NADA packages to CVM and received one response as follows:

Copper sulfate submission. Environmental assessment: effect of copper sulfate used as a therapeutant in aquaculture. December 20, 1996.

Formalin, CVM response. Based in part on the December 15, 1995 submission from UMSC on the safety of formalin treatments on fish eggs, the CVM placed a notice in the October 18, 1996 issue of the Federal Register inviting sponsors of formalin to amend their labels to include the extended claims for both the fungicide uses (based on UMSC studies) and parasiticide uses (based on studies at Auburn University, Auburn, AL).

Hydrogen peroxide submission. Schnick, R.A., and J.J. Rach. 1996. Summary of data and information on the use of hydrogen peroxide to control bacterial infections and external parasite infestations on freshwater fish. Submitted to the Center for Veterinary Medicine, Rockville, MD. August 27, 1996. 20 pp.

Current Status: The UMSC and SNARC were pleased with CVM's decisions to extend 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 and the agency's the lack of concern over human food or environmental safety of copper sulfate making for a fairly easy NADA approval.

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APPENDIX I: ACCUMULATED PRODUCTS OF THE "APPROVAL OF DRUGS FOR PUBLIC FISH PRODUCTION PROJECT," A PROJECT OF THE INTERNATIONAL ASSOCIATION OF FISH AND WILDLIFE AGENCIES

NADA SUBMISSIONS

Benzocaine submission. Stehly, G.R., J.R. Meinertz, W.H. Gingerich, P.M. Mazik, and M.P. Gaikowski. 1995. Effects of temperature on the loss of benzocaine and acetylated benzocaine residues from edible tissues of channel catfish (*Ictalurus punctatus*). Contract completion report for Interagency Agreement Number 224_92_7036. Submitted to the Center for Veterinary Medicine, Rockville, MD, October 5, 1995. 121 pp. Accepted by CVM on November 5, 1995.

Benzocaine submission. Stehly, G.R., J.R. Meinertz, J.A. Bernardy, and W.H. Gingerich. 1995. Final report for contract study entitled "Effects of temperature on the pharmacokinetics of benzocaine in rainbow trout *Oncorhynchus mykiss*, Part I: Development and validation of experimental methods and Part II: Pharmacokinetic profiles of benzocaine in temperature-acclimated fish." U.S. Food and Drug Administration, Contract number FDA 224_92_7036. Submitted to the Center for Veterinary Medicine, Rockville, MD. May 11, 1995. 193 pp. Accepted by CVM June 22, 1997.

Chloramine-T submission. Dawson, V.K., W.H. Gingerich, L.J. Delaney, P.L. Vetter, and M.J. Rogazcewski. 1995. Isolation and characterization of chloramine-T metabolites in rainbow trout after use-pattern treatment with [^{14}C]chloramine-T. Submission of metabolite identification data to support the approval of chloramine-T to control mortalities associated with external flavobacterial infections in freshwater fish. Submitted to the Center for Veterinary Medicine, Rockville, MD, April 13, 1995. 100 pp. Accepted by CVM on July 20, 1995.

Chloramine-T submission. Dawson, V.K. and W.H. Gingerich. 1991. Accumulation and clearance of chloramine-T residues in rainbow trout after use-pattern treatment with [^{14}C]chloramine-T. Submission of total residue depletion data to support the approval of chloramine-T to control mortalities associated with external flavobacterial infections in freshwater fish. Submitted to the Center for Veterinary Medicine, Rockville, MD. April 15, 1991. 250 pp. Accepted by CVM on July 20, 1995.

Chloramine -T, CVM response. The CVM responded on July 20, 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 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. Environmental assessment: effect of copper sulfate used as a therapeutant in aquaculture. Submitted to the Center for Veterinary Medicine, Rockville, MD. December 20, 1996. 20 pp.

Copper sulfate submission. Hobbs, M., F. Kadlubar, D. Schlenk, B.R. Griffin, and C.D. Brand. 1996. Technical report for Experiment No. E06897.01 Accumulation of copper in edible muscle of channel catfish (*Ictalurus punctatus*) following exposure to waterborne copper sulfate. Submitted to the Center for Veterinary Medicine, Rockville, MD. April 4, 1996. 20 pp. Accepted by CVM June 1, 1996.

Copper sulfate, CVM response. The 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.

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Formalin submission. Rach, J.J., G.E. Howe, and T.M. Schreier. 1995. Safety of formalin treatments on fish eggs. Final report submitted to the Center for Veterinary Medicine, Rockville, MD. December 15, 1995. 128 pp. Accepted by CVM October 18, 1996.

Formalin, CVM response. The CVM placed a notice in the October 18, 1996 issue of the Federal Register inviting NADA sponsors of formalin to amend their labels to include the extended claims for both the fungicide uses (based on UMSC studies) and parasiticide uses (based on studies at Auburn University, Auburn, AL).

Hydrogen peroxide submission. Schnick, R.A., and J.J. Rach. 1996. Summary of data and information on the use of hydrogen peroxide to control bacterial infections and external parasite infestations on fresh water fish. Submitted to the Center for Veterinary Medicine, Rockville, MD. August 27, 1996. 20 pp.

Hydrogen peroxide submission. Rach, J.J., 1996. Regarding an amendment to the low regulatory priority ruling of hydrogen peroxide. Submitted to the Center for Veterinary Medicine, Rockville, MD. February 29, 1996. 6 pp.

Hydrogen peroxide submission. Schnick, R.A., L.L. Marking, J.J. Rach and T.M. Schreier. 1993. Request for determination of regulatory status of hydrogen peroxide. Submitted to the Center for Veterinary Medicine, Rockville, MD. August 6, 1993. 6pp + attachments. Accepted by CVM January 11, 1994.

Hydrogen peroxide, CVM response. The CVM concluded on January 11, 1994 that "hydrogen peroxide, used at 250-500 mg/L to control fungi on all species and life stages of fish, including eggs, will be considered a new animal drug of low regulatory priority."

Oxytetracycline submission. Stehly, G.R., J.R. Meinertz, and W.H. Gingerich. 1996. High performance liquid chromatography (HPLC) method for oxytetracycline in fish proposed for use in bridging studies with the official microbiological method. Preliminary report submitted to Dr. P. Chu, Center for Veterinary Medicine. June 17, 1996. 48 pp. Accepted by CVM November 25, 1996.

Oxytetracycline Response. The CVM chemist reviewing the HPLC method, forwarded a recommendation on November 25, 1996 that the method was acceptable for use in a bridging study between the HPLC method and the official microbial inhibition assay. Accepted by CVM November 1996.

STUDY PROTOCOLS

Dawson, V.K., R.A. Schnick, and L.J. Delaney. 1993. Study protocol for a compassionate aquaculture Investigational New Animal Drug (INAD) for chloramine-T. Submitted to the Center for Veterinary Medicine, Rockville, MD. March 31, 1993. 51 pp. [Template for all compassionate INADs].

Gingerich, W.H. and G.R. Stehly. 1994. Effects of temperature on the loss of benzocaine and acetylated benzocaine residues from the edible tissues of channel catfish (*Ictalurus punctatus*). UMSC Study Protocol CAP-94-00078-01. Approved by UMSC Director on March 11, 1994. 52 pp.

Gingerich, W.H. 1993. Effects of temperature on the pharmacokinetics of benzocaine in rainbow trout, *Oncorhynchus mykiss*, Part II: Pharmacokinetic profiles of benzocaine in

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- Griffin, B.R., F. Kadlubar, J. Gollon, and M.S. Hobbs. 1995. Experiment No. E06955.01 Accumulation of manganese in edible muscle of channel catfish (*Ictalurus punctatus*) following exposure to waterborne potassium permanganate. Study protocol accepted by the Center for Veterinary Medicine, Rockville, MD. September 13, 1995.
- Hobbs, M., F. Kadlubar, D. Schlenk, B.R. Griffin, and C.D. Brand. 1994. Project # E06897.01 Accumulation of copper in edible muscle of channel catfish (*Ictalurus punctatus*) following exposure to waterborne copper sulfate. Study protocol accepted by the Center for Veterinary Medicine, Rockville, MD. September 30, 1994. 22 pp.
- Howe, G.E. 1996. A method for inducing saprolegniasis in rainbow trout. UMSC study protocol CAP-96-00048-3. Approved by UMSC Director on October 15, 1996. 24 pp.
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- Rach, J.J. 1996. Toxicity of hydrogen peroxide to eggs and fry of cold-, cool-, and warmwater fish species
- Rach, J.J. 1995. Inducing fungal infections on fish. UMSC protocol TOX-95-00048-6. Approved by UMSC Director on July 26, 1995. 10 pp.
- Rach, J.J. 1995. Safety of hydrogen peroxide treatments on fish eggs. UMSC study protocol TOX-95-00048-7. Approved by UMSC Director on March 24, 1995. 14 pp.
- Rach, J.J. 1994. Toxicity of hydrogen peroxide to salmonid eggs and fry. UMSC study protocol TOX-94-00048-4. Approved by UMSC Director on October 24, 1994. 13 pp.
- Rach, J.J. 1994. Toxicity of hydrogen peroxide to rainbow trout eggs at different developmental stages. UMSC study protocol TOX-94-00048-3. Approved by UMSC Director on September 15 1994. 13 pp.
- Rach, J.J. 1994. Safety of formalin treatments of fish eggs. UMSC study protocol TOX-94-00048-2. Approved by UMSC Director on April 6, 1994. 14 pp.
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Stehly, G.R., J.R. Meinertz, and W.H. Gingerich. 1995. Effects of temperature on the loss of benzocaine and acetylated benzocaine from the edible tissues of the rainbow trout, *Oncorhynchus mykiss*. UMSC study protocol. Approved by UMSC Director on February 2, 1995. 51 pp.

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- Gingerich, W.H., J.R. Meinertz, V.K. Dawson, J.E. Gofus, L.J. Delany, and P.R. Bunnell. 1995. Distribution and elimination of [¹⁴C]sarafloxacin hydrochloride from tissues of juvenile channel catfish (*Ictalurus punctatus*). *Aquaculture* 131:23_36.
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- Schnick, R.A. and R.D. Armstrong. (In press). Aquaculture drug approval progress in the United States. *Northern Aquaculture*.
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Dawson, V.K., W.H. Gingerich, G.E. Howe, J.J. Rach, R.A. Schnick, G.R. Stehly, and B.R. Griffin. 1996. Approval of drugs for public fish production: second mid-year report of progress. National Biological Service, Upper Mississippi Science Center, La Crosse, WI. January 1996. 17 pp.

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Hobbs, M.A. 1996. The distribution and elimination of the herbicide copper sulfate in fingerling channel catfish (*Ictalurus punctatus*). Master of Science Thesis, Department of Pharmacology, University of Arkansas for Medical Sciences. Little Rock, AR. 90 pp.

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- Schnick, R.A. 1996. Aquaculture drugs (INADs/NADAs): progress report for the period September 1, 1995 to August 31, 1996. Submitted to the Director, North Central Regional Aquaculture Center, Michigan State University, East Lansing, MI. November 12, 1996. 8 pp.
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- Gingerich, W.H. and G.R. Stehly. 1994. Effects of temperature on the loss of benzocaine and acetylated benzocaine residues from edible tissues of channel catfish, *Ictalurus punctatus*. Revised research protocol sent to the Office of Science, Center for Veterinary Medicine, Rockville, MD. March 11, 1994. 55 pp. Proposal approved.
- Schnick, R.A., W.H. Gingerich, G.R. Stehly, V.K. Dawson, and B.R. Griffin. 1994. Approval of drugs for public fish production. Revised research proposal submitted to the ad hoc Committee on Aquaculture, International Association of Fish and Wildlife Agencies, March 9, 1994. 98 pp. Proposal approved for 5 years.
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- Stehly, G.R. 1994. Analytical method development and residue depletion studies for approval of sarafloxacin in salmonids. Proposal for Fisheries Federal Aid Administrative Monies for FY95. June 29, 1994, 34 pp.
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- Dawson, V.K., J.J. Rach, and T.M. Schreier. 1995. Fungus control in fish culture using hydrogen peroxide. Presented at Aquaculture '95, San Diego, CA, February 1-4, 1995.
- Dawson, V.K. and W.H. Gingerich. 1994. Comparison of accumulation and clearance rates of chloramine_T residues between fingerling and juvenile rainbow trout after use_pattern treatment with [ring UL_14C]chloramine_T. Presented at the 10th Annual Meeting of the Aquaculture Association of Canada, Charolettetown, Prince Edward Island, Canada, August 23_27, 1994.
- Dawson, V.K. 1994. Isolation and characterization of metabolites of the disinfectant chloramine_T. Invited paper presented as part of the Agrochemical Metabolism Symposium at the 207th American Chemical Society National Meeting, San Diego, CA, March 13_17, 1994.
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- Gingerich, W.H. 1996. Update on the progress of the project "Approval of Drugs for Public Aquaculture". Presented at the meeting of the Working Group on Quality Assurance in Aquaculture Production, Arlington, TX, February 14, 1996.
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- Howe, G.E., J.J. Rach, T.M. Schreier. 1995. Treating fish diseases with hydrogen peroxide. Presented at the Midwest Fish and Wildlife Conference, Detroit, MI, December 6, 1995.
- Howe, G.E. 1995. An overview of a compassionate aquaculture INAD for formalin use as a fungicide. FWS/INAD Coordination Workshop, Bozeman, MT, August 1-4, 1995.
- Howe, G.E., J.J. Rach, T.M. Schreier. 1995. Co-lectured training session entitled "Therapeutic uses of chemicals in aquaculture". Presented at the Leetown National Education Training Workshop Use of Drugs and Chemicals in Aquaculture, La Crosse, WI, April 4-6, 1995.
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- Rach, J.J., M.P. Gaikowski, and J.J. Olson. 1996. Importance of analytically evaluating chemical treatments. Presented at the Midwest Fish and Wildlife Conference, Omaha, NE, December 8-11, 1996.
- Rach, J.J. Evaluation of hatchery hydrogen peroxide treatments. 1996. Presented at the Wisconsin Department of Natural Resources Fisheries Production Annual Meeting, Stevens Point, WI, August 22, 1996.
- Rach, J.J., M.P. Gaikowski, and J.J. Olson. 1996. Hydrogen peroxide research developments. Presented at the American Fisheries Society_Fish Health Section meeting, Madison, WI, August 6-9, 1996.
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- Rach, J.J., T.M. Schreier, and S.D. Redman. 1995. Hydrogen peroxide: A promising new fish therapeutant. Presented at Chemistry in Aquaculture Symposium, Western Carolina University, Cullowhee, NC, May 31-June 4, 1995.
- Rach, J.J., V.K. Dawson, and S.D. Redman. 1995. Use of hydrogen peroxide in aquaculture. Presented at the 3rd Annual New England Farmed Fish Health Workshop, East Port, ME, April 7-8, 1995.
- Rach, J.J., T.M. Schreier, and G.E. Howe. 1995. Therapeutic uses of chemicals in aquaculture. Presented at the Leetown National Education Training Workshop Use of Drugs and Chemicals in Fish Culture, La Crosse, WI, April 4-6, 1995.
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- Rach, J.J., and T.M. Schreier. 1994. Research on promising fish therapeutants. Presented at the Wisconsin Department of Natural Resources, Fisheries Production Annual Meeting, La Crosse, WI, August 17-18, 1994.
- Schleis, S.M., G.R. Stehly, J.R. Meinertz, and W.H. Gingerich. 1996. Determination of benzocaine and acetylated benzocaine residues in edible tissues of channel catfish (*Ictalurus punctatus*). Presented at the La Crosse-Winona Section of the American Chemical Society Meeting in Miniature, La Crosse, WI, December 5, 1996.
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- Schnick, R.A. 1996. Overview of NADA Coordinator activities, International Project update, short-term INAD/NADA needs. Presented at the FWS INAD Coordination Workshop, Bozeman, MT, August 14-15, 1996.
- Schnick, R.A. 1996. Cooperative fish therapeutic funding initiative--States in partnership with Federal agencies to ensure the future of public fish culture. Presented at the 61st North American Conference on Wildlife and Natural Resources, Tulsa, OK, March 24-28, 1996.
- Schnick, R.A. 1996. Report on progress and research study objectives of the Federal-State Drug Registration Partnership. Presented at the meeting of the International Association of Fish and Wildlife Agencies, ad hoc Committee on Aquaculture, Tulsa, OK, March 24, 1996.

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- Schnick, R.A. 1996. Aquaculture drugs. Presented at the 1996 Program Planning Meeting and Program Review. North Central Regional Aquaculture Center, East Lansing, MI, February 24, 1996.
- Schnick, R.A. 1996. Proper use of fish therapeutants based on legal requirements-gill lice, bacterial gill disease, furunculosis, etc. Presented to the annual meeting of the Michigan Aquaculture Association, East Lansing, MI, February 23, 1996.
- Schnick, R.A. 1996. National Aquaculture NADA Coordinator update. Presented at the Working Group on Quality Assurance in Aquaculture Production, Arlington, TX, February 14, 1996.
- Schnick, R.A. 1996. INAD/NADA update. Presented at the Western Regional Aquaculture Expo '96, Sacramento, CA, February 7-9, 1996.
- Schnick, R.A. 1996. Status of aquaculture INADs and NADAs. Presenter and coordinator, Midcontinent Warmwater Fish Culture Workshop and INAD/NADA Coordination Meetings, Council Bluffs, IA, February 6-8, 1996.
- Schnick, R.A. 1995. INAD\NADA Coordinators Workshop under the sponsorship of the Center for Veterinary Medicine, organizer and presenter, Rockville, MD, November 1-2, 1995.
- Schnick, R.A. 1995. Activities of the National Coordinator for Aquaculture New Animal Drug Applications. Presented at the Annual meeting of the National Research Support Program Number 7 (NRSP-7), Rockville, MD, October 2, 1995.
- Schnick, R.A. 1995. Funding crisis for drugs/therapeutants and coordination of aquaculture INADs and NADAs. Annual meeting of the U.S. Trout Farmers Association, Twin Falls, ID, September 27-30, 1995.
- Schnick, R.A. 1995. FWS/INAD Coordination Workshop, presenter and coordinator, Bozeman, MT, August 1-4, 1995.
- Schnick, R.A. 1995. Priority list of aquaculture drugs. Presented at Working Group on Quality Assurance in Aquaculture Production, Washington, DC, June 23, 1995.
- Schnick, R.A. 1995. Session III: Pharmacology & Biotechnology. Convener and Presenter at the Chemistry in Aquaculture Symposium, Cullowhee, NC, May 31-June 2, 1995.
- Schnick, R.A. 1995. The status of NADA (New animal drug approvals). Presented at the Idaho Aquaculture Association annual meeting, Twin Falls, ID, May 19-22, 1995.
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- Schnick, R.A. 1994. Approval of drugs and chemicals for use by the aquaculture industry. Presented at the Seventh NRSP-7/FDA Workshop for Minor Use Drugs: Drugs in Aquaculture: Current Status - Future Goals, Bethesda, MD, September 29-30, 1994.
- Schnick, R.A. 1994. Review of drugs currently used in fish health. Course, "Pharmacology for Fish Health Biologists," La Crosse, WI, July 28, 1994.
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- Schnick, R.A. 1994. Chloramine-T protocol review. Presented at the U.S. Fish and Wildlife Service INAD Training Session, Denver, CO, May 17, 1994.
- Schnick, R.A. 1994. Drug/chemical use in fisheries: registration and availability of compounds. Presented at the Fisheries Operation (Module II), Office of Technical Fisheries Training, Kearneysville, West VA, April 15, 1994.
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- Schreier, T.M., J.J. Rach, V.K. Dawson, and G.E. Howe. 1995. Development of hydrogen peroxide for fish culture. Presented at the Wisconsin chapter meeting of the American Fisheries Society, Madison, WI, January 18, 1995.
- Schreier, T.M., and J.J. Rach. 1994. Methods of evaluating fish therapeutants. Presented at the Wisconsin Department of Natural Resources Fisheries Production Annual Meeting, La Crosse, WI, August 17-18, 1994.
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- Stehly, G.R. 1994. Pharmacokinetics, drug administration, drug elimination, metabolism and excretion. Presented at the Leetown National Education Training Center Course Pharmacology for Fish Health Biologists, La Crosse, WI, July 27-28, 1994.
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